Assortative Mating in Zebra Finch Subspecies, Taeniopygia guttata guttata and T. g. castanotis

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Assortative mating in zebra finch subspecies, *Taeniopygia guttata guttata* and *T. g. castanotis*

N. S. CLAYTON

Edward Grey Institute of Field Ornithology, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

CONTENTS

SUMMARY

There are two subspecies of the zebra finch, *Taeniopygia guttata guttata* and *Taeniopygia guttata castanotis*. *T. g. guttata* is found on the Lesser Sunda islands in Indonesia and the males differ from their Australian counterparts, *T. g. castanotis*, in having a thinner breast-band and grey chin and throat instead of the black and white throat bars. The songs of male *guttata* are longer and sung at a higher frequency than those of male *castanotis*. In contrast to the substantial differences between the two subspecies, there is little geographic variation with subspecies.

In a recent aviary study of the social interactions and pair formation between members of captive colonies of *guttata* and *castanotis*, members of the two subspecies were observed to mate assortatively, i.e. *guttata* and *castanotis* did not form mixed pairs (Böhner *et al.* 1984). This raises the question of which cues ensure that the two subspecies are behaviourally isolated and hence mate assortatively. In song playback experiments, females of both subspecies discriminated between *guttata* and *castanotis* songs, preferring the songs of males of their own subspecies. In multiple mate choice tests and observations of the same individuals during pair formation in aviaries, male and female *guttata* and *castanotis* were found to prefer members of their own subspecies. However, *guttata* males that were painted to resemble *castanotis* males were preferred by *castanotis* females over unpainted *guttata* males whereas *guttata* females preferred the unpainted *guttata* males. In the aviary, the *castanotis* females paired with painted *guttata* males and *guttata* females paired with unpainted *guttata* males.

These findings suggest that differences between the two subspecies in both song and breast-band size could play a role in mate choice and subspecies discrimination, thus leading to assortative mating between the two subspecies in captivity. To assess the importance of early rearing experience on the development of these visual and vocal differences between the two subspecies and its effect on the development of sexual preferences, *guttata* and *castanotis* that had been cross-fostered to the other subspecies were compared with those that has been normally raised by members of their own species.

When cross-fostered to the other subspecies, *castanotis* and *guttata* males resembled their own subspecies in the macrostructural features of song which distinguish the two subspecies' songs. Hybrid males that were raised by one *guttata* and one *castanotis* parent have songs that are intermediate between those of *guttata* and those of *castanotis* males. These results suggest that rearing experience has little, if any, effect on the development of these macrostructural song differences between the two subspecies. In playback experiments, females preferred the songs of their foster-father's subspecies, irrespective of whether the songs were from males that had been cross-fostered or normally raised. This indicates that females use these macrostructural differences in song for subspecies discrimination and that female song preferences are learnt.

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Cross-fostered zebra finches resemble normally raised members of their own subspecies in size and plumage, and hybrids were intermediate. In multiple mate choice tests, females preferred normally raised birds of their own subspecies over those that had been cross-fostered and over those of the other subspecies. Since cross-fostered males do not appear to differ from normally raised birds of the same subspecies in plumage, size or song, these results suggest that females may discriminate between *guttata* and *castanotis* males on the basis of behavioural cues.

Studies of multiple mate choice and pair formation showed that when both sexes were cross-fostered to the other subspecies mixed pairs (*guttata–castanotis*) were formed, suggesting that early experience with the foster-parents can have an influence on pair formation through sexual imprinting on the parents. However, when one sex is normally raised and the other is cross-fostered, the cross-fostered birds usually pair with members of their own subspecies. This indicates that the likelihood of pairing with the 'wrong' subspecies is reduced when cross-fostered individuals interact with members of their own subspecies. This would provide an adaptive mechanism for maintaining behavioural isolation between the two subspecies. Comparing the mate choice during one-way and two-way interaction suggests that normally raised zebra finches choose more often than cross-fostered birds and that, when both sexes are cross-fostered, the prerogative lies with the female.

1. INTRODUCTION

An emerging question in ethology concerns the link between the development and function of behaviour. One aspect of this which is of great interest is mate choice and, in particular, the relationship between the development and function of sexual preferences.

Mate choice can be defined as any behaviour pattern, shown by members of one sex, that leads to their being more likely to mate with particular members of the opposite sex (Halliday 1983). There are good reasons for believing that animals should choose a partner rather than mating indiscriminately. Given that individuals differ in their quality as potential mates, natural selection should favour mechanisms that ensure that individuals choose partners in such a way as to maximize their mating success.

It is generally assumed that females are the 'choosy sex' because each egg represents a relatively large proportion of a female's lifetime production of gametes when compared with a sperm. However, in zebra finches *Taeniopygia guttata*, and in the majority of birds and other species in which both sexes care for the young, male reproductive success is not determined only by the number of females inseminated but also depends on the amount of parental care invested in the offspring. As a result, in species with biparental care males should also be 'choosy'.

One of the most problematic issues concerns how individuals assess the quality of potential mates. Unlike females of species in which males contribute only gametes, in species with biparental care males and females should exercise some degree of choice and they can use both genetic and nongenetic cues. There are three basic ways in which an individual might choose a mate. First, a bird may select a mate on the basis of the material resources that individual can offer. For example, female great tits *Parus major* appear to choose males who provide higher quality resources for their offspring. Since the males help with feeding and nest defense, the female can increase her reproductive success by producing more offspring that survive (Norris 1990). Alternatively, an individual may select a mate with 'good genes', i.e. ones that, when transmitted to the offspring, will increase the offspring's probability of survival. For example, Partridge (1980) found that the offspring of female fruitflies *Drosophila* that were allowed to mate freely with a population of males outcompeted the offspring of females that were forced to mate with a particular partner. Therefore, by choosing mates, females can increase the survival of their offspring, who have inherited their father's good genes. Since male fruitflies contribute only deoxyribonucleic acid (DNA) to their offspring, the female must somehow be choosing the males on the basis of their good genes. Thirdly, individuals might choose a mate that will enable them to produce a higher proportion of 'sexy' sons, i.e. sons that are more likely to be chosen as mates or are more likely to mate with highly fertile females (Weatherhead & Robertson 1979).

Whatever the mechanism, to ensure viable young, the first, vital step is to select a mate of the correct species. Where there is more than one form of the species, assortative mating may occur, whereby individuals show a significant tendency to mate with other individuals of either a similar (positive assortative mating) or dissimilar (negative assortative mating) phenotype to themselves (for a review see Bateson (1983)).

Preferences for certain plumage types can lead to assortative mating. An example of this is seen in the lesser snow goose, *Anser caerulescens*. There are two colour morphs, blue and white. Cooke and his coworkers (Cooke *et al.* 1976; Cooke 1978) found that about 90% of the geese pair with a partner of the same colour as themselves (positive assortative mating), as a result of a preference developed early in life to pair with a mate that is the same colour as their parents. Similar effects of assortative mating by plumage colouration have been described for domestic fowl (Lill & Wood-Gush 1965; Lill 1968*a*, *b*) and for captive mallard ducks (Cheng *et al.* 1978).

Vocal differences between populations within a species or closely related group of species can also lead to assortative mating. In some species of frogs, for example, individuals mate assortatively according to the pulse-repetition or trill rate of the calls (see

Gerhardt 1988). In song birds, there is often considerable geographic variation in song within a species. For example, male swamp sparrows, *Melospiza melodia*, from New York and northern Minnesota differ in their song syntax and both males and females can discriminate between the two song dialects (Balaban 1988*a*). In swamp sparrows, assortative mating might occur as a result of females pairing with a male whose song is characteristic of their natal area (Balaban 1988*b*).

In these examples the adaptive significance of assortative mating is not at all clear. Published data showing an advantage of assortatively mated pairs over non-assortatively mated pairs is lacking for the frog and sparrow studies. In the snow goose, assortatively mated pairs do not appear to have a reproductive advantage over non-assortatively mated pairs for most fitness components related to fecundity and viability. Indeed, in the one component where a significant difference was found, positive assortative mating appeared to decrease fertility since mixed pairs enjoyed consistently better nesting success than pure pairs (Findlay *et al.* 1985). However, in the three-spined stickleback, *Gasterosteus aculeatus*, in Canada there is assortative mating between the anadromous and the fresh water ecotypes which appears to have an obvious adaptive value: hybrid progeny are unlikely to be as well adapted to either ecotype as the pure-bred progeny (Hay & McPhail 1975). Unfortunately, there are no empirical data to support this prediction.

The major part of this paper will be concerned with assortative mating in zebra finches. Four issues will be discussed. First, I shall consider the differences between the two zebra finch subspecies, *T. g. guttata* (Vieillot) and *T. g. castanotis* (Gould), notably in size, plumage and vocalizations, which might be involved in subspecies recognition. Secondly, I shall describe some experiments that show that females prefer and recognize males of their own subspecies on the basis of song and that subspecies differences in male plumage characteristics can also be used by females in choosing their mates, leading to assortative mating between *guttata* and *castanotis*. The third issue is a developmental one, concerning the extent to which these preferences are governed by early rearing experience. Finally, I shall attempt to link the development and function of assortative mating between the two subspecies by considering the relationship between the development of subspecies recognition in zebra finches and the function of sexual preferences for members of their own subspecies.

The zebra finch is a particularly good species for studying these questions for three reasons. The two subspecies differ in their geographic distribution in the wild: *guttata* occur only on the Lesser Sunda islands in Indonesia whereas *castanotis* are found on the Australian mainland, 400 km to the northwest. The two subspecies are largely geographically isolated, although there may be a zone of overlap on the remote islands west of Timor (see figure 1). In captivity the birds are capable of producing fertile, viable hybrids, whose offspring can reproduce (N. S. Clayton, personal observation), suggesting that the two forms should be classified as subspecies (Clayton *et al.* 1990). None the less, when captive colonies of the two subspecies are housed together in aviaries, *guttata* and *castanotis* show a significant tendency to pair with members of their own subspecies (Böhner *et al.* 1984; Clayton 1990*a*). This suggests that behavioural differences between the two subspecies in their sexual preferences could, at least potentially, effect assortative mating, leading to total reproductive isolation, and thus speciation, between *guttata* and *castanotis* (Mayr 1963, 1970).

A second advantage of working with zebra finches is that the two subspecies have remarkably similar breeding ecologies and behaviour but show discrete, quantitative differences in their size, plumage and vocalizations. This means that each parameter can be varied in turn to assess which of these potential cues might play a role in assortative mating between the two subspecies. Furthermore, the subspecies differences in plumage (bill colour and male breast-band) can be manipulated by painting the birds, providing a verification of the importance of plumage cues. Since the vocalizations (distance calls and song) that differ between *guttata* and *castanotis* could be those which are learnt, it should be possible to obtains birds that visually resemble their own subspecies but produce the vocalizations of the other. This involves the technique of cross-fostering: in this case the young of *guttata* are transferred as eggs to incubating pairs of the *castanotis*, whose own eggs are transferred to incubating pairs of *guttata*. By using cross-fostered birds, it was hoped to be able to ascertain the relative importance of plumage and song for assortative mating between the two subspecies. Sexual preferences in birds are thought to emerge largely as a result of sexual imprinting, the process by which young birds learn (sub)species-specific characteristics that enable them to find a mate of their own subspecies when adult (see, for example, Immelmann (1972)). By comparing cross-fostered zebra finches of both subspecies with those that had been normally raised by members of their own subspecies, I wanted to examine the extent to which both song and mate preferences are influence by early rearing experience.

The third justification for choosing the zebra finch is for the simple reason that it is one of the most commonly used species in laboratory studies of the development of both song and mate preferences and it is the only species in which a relationship between the two has been investigated (Clayton 1989*a*). The practical explanations for this are that the zebra finch is a widely available cagebird which can easily be bred in a small cage, is cheap to feed, readily breeds throughout the year in captivity and has a generation time of about only 90 days.

The zebra finch has proved to be an ideal subject for studying behavioural development. The time is now ripe to link what is known about the development of song and mate preferences with their possible function in assortative mating between the two subspecies. The first step in this link is to detail the qualitative and quantitative differences between *guttata* and *castanotis*.

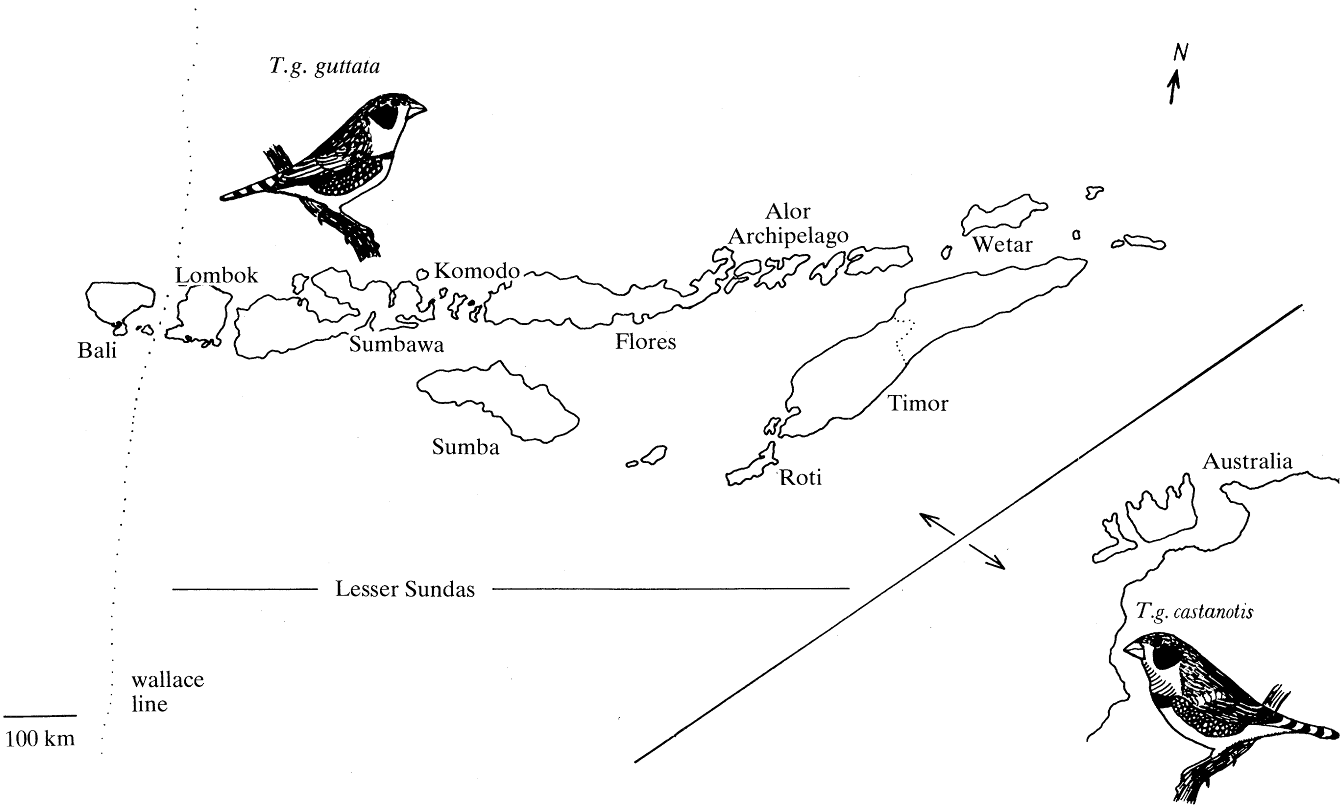

Figure 1. The distribution of the two subspecies of zebra finch, *Taeniopygia guttata*. *T. g. guttata* occurs on the Lesser Sunda islands in Indonesia to the east of the Wallace Line (from Lombok to Timor). *T. g. castanotis* is found on the Australian mainland.

2. DIFFERENCES BETWEENN *T. g. guttata* AND *T. g. castanotis*

Zebra finches are highly social, sexually dimorphic, monogamous estrildid finches. They breed in loose colonies in response to rainfall which is essential if there is to be sufficient food for feeding the young. Rain is unpredictable in some parts of the range, which explains why these birds are ready to breed all year round. Appropriate breeding conditions are not only unpredictable, but also brief and transient, although maintaining a stable monogamous pair bond means that reproduction can begin more or less as soon as the rains start (Immelmann 1962).

The Australian zebra finch, *T. g. castanotis*, is found throughout most of the Australian mainland, particularly in savannah, woodlands, grasslands and in parts of the central desert where shrubs provide roosting and nesting sites and there is surface water for drinking (Immelmann 1962). Keast (1958) found little plumage variation between *castanotis* from different localities within Australia and suggested that this lack of geographic variation might be the result of periodic droughts in inland Australia, which force the birds to the remaining areas of water, leading to some intermixing of stocks from different areas. Keast also noted considerable individual variation in bill colour and in the size of the black breast-band of males. In addition, he found a south–north cline of gradually decreasing size, with birds from the southern most areas being as much as 5 % larger than those from northern Australia.

The *guttata* subspecies is found on a chain of islands in Indonesia, the Lesser Sundas, which lie about 400 km to the northwest of the Australian mainland (figure 1). They have been recorded on Lombok, Sumbawa, Sumba, Komodo, Flores, Timor and Wetar (Keast 1958; Clayton *et al.* 1990).

To examine the geographical variation in zebra finches Richard Zann, Dave Hodson and I compared size, plumage and vocalizations of live, wild-caught and captive *guttata* and *castanotis* (Clayton *et al.* 1990). Data were also collected from free living *castanotis* at Wungnu, Victoria and from island populations of *guttata* on the Lesser Sunda islands, Lombok, Sumbawa, Flores and Timor.

Guttata and *castanotis* differed in five respects:

(*a*) *Castanotis* were significantly larger as measured by wing length, weight, bill length and bill depth (figure 2).

(*b*) Munsell colour chips were used to rank the upper mandible of the bill according to redness, darkness and brightness (see Burley & Coopersmith 1987; Clayton *et al.* 1990). *Castanotis* had redder, darker, brighter bills than *guttata*, with males having redder bills than females (U tests, $p < 0.001$ for all comparisons for males, $p > 0.05$ for females, having confirmed for overall heterogeneity with the Kruskal Wallis H test).

(*c*) Only male zebra finches posess a black breast-band and this, together with the absence or presence of

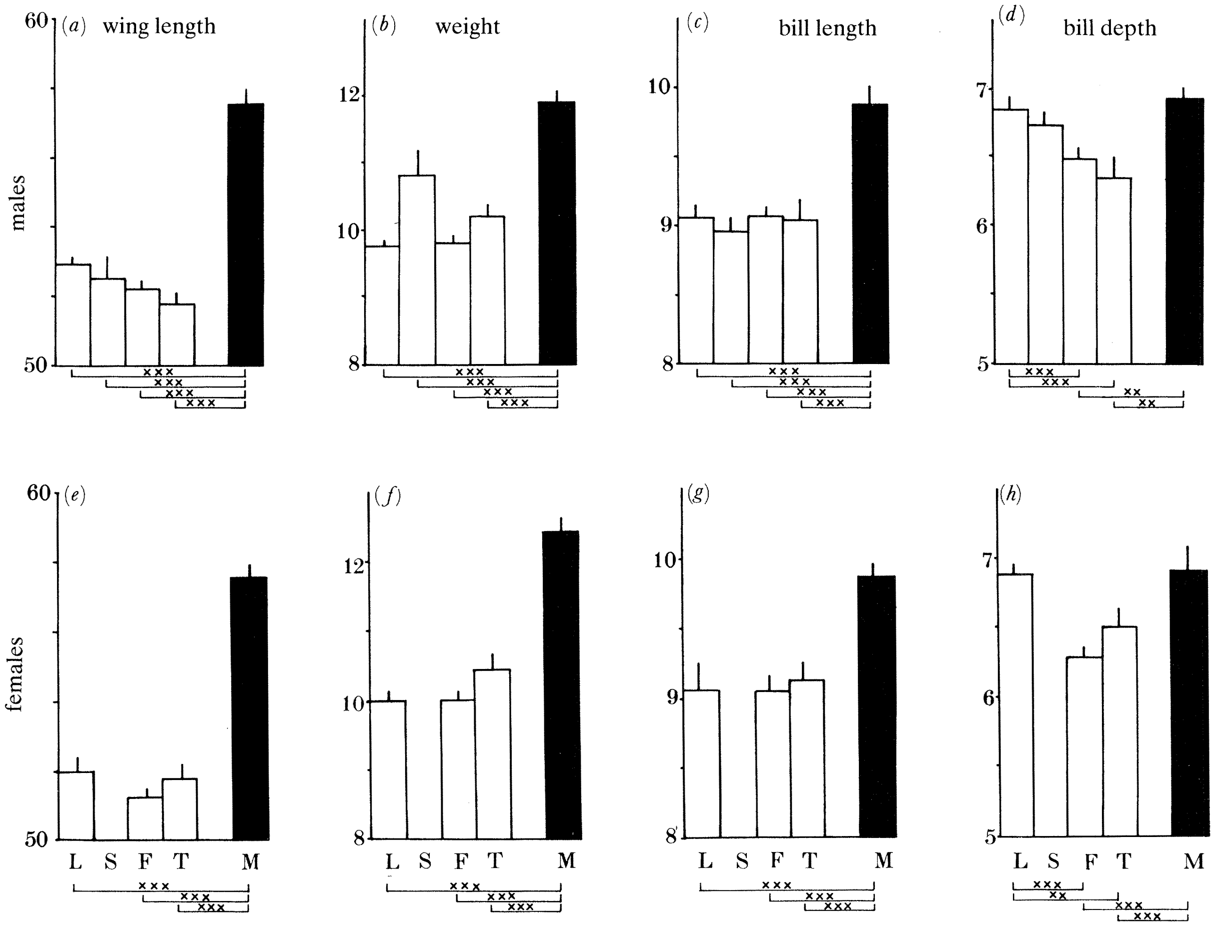

Figure 2. Histograms comparing free-living *guttata* (open bars) on Lombok (L; 11 males, 5 females), Sumbawa (S; 4 males), Flores (F; 12 males, 14 females) and Timor (T; 8 males, 5 females) and *castanotis* (dark bars) in Victoria (M; 55 males, 45 females) showing mean ± s.d. in wing length (maximum wing chord in millimetres), weight (in grammes), bill length (in millimetres, from the tip of the bill to the naso-frontal joint) and bill depth at the nares (in millimetres, perpendicular to the commissure at the anterior edge of the nares). Overall heterogeneity was confirmed using one-way analyses of variance (ANOVA; $p < 0.001$ for all eight tests). Individual groups were compared with T tests *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

fine black barring on the throat and upper breast, is the most striking plumage difference between males of the two subspecies. *Castanotis* males had larger black breast-bands than those of *guttata* males and they had strongly pronounced black barring on the throat and upper breast which was absent from *guttata* males (Mann Whitney U tests, $p < 0.001$ for all comparisons, having confirmed overall heterogeneity with the Krustal Wallis H test).

(*d*) *Castanotis* produced lower frequency distance calls and the males differed from *guttata* males and females of both subspecies in incorporating a noise element into the distance call in addition to the tonal element which is present in the distance call of all zebra finches (figure 3).

(*e*) In both subspecies only males sing. *Castanotis* songs are shorter than *guttata* songs, with fewer elements per phase, and sung at a lower frequency (figure 4).

These subspecies differences are particularly striking in view of the lack of geographic variation within Australia (Keast 1958) and the Lesser Sunda islands. Although clines of decreasing size are found in wing length (Australia: south–north) and bill depth (Lesser Sundas: west–east). Clayton *et al.* (1990) argue that the differences are not sufficiently large to merit the splitting of *guttata* into separate subspecies according to island.

The differences between *guttata* and *castanotis* are probably the result of geographic isolation between the two forms. Given that the two subspecies mate assortatively according to subspecies in captivity (Böhner *et al.* 1984), the results in this section suggest that the assortative mating might be mediated by differences in size, plumage or vocalizations between *guttata* and *castanotis*. The next section addresses the importance of these differences for assortative mating between zebra finch subspecies.

3. WHICH CUES ENSURE THAT THE TWO SUBSPECIES MATE ASSORTATIVELY?

(*a*) *Vocalizations: song*

Given that song forms an integral part of the courtship ceremony performed by male zebra finches, and that *guttata* and *castanotis* males differ in their song,

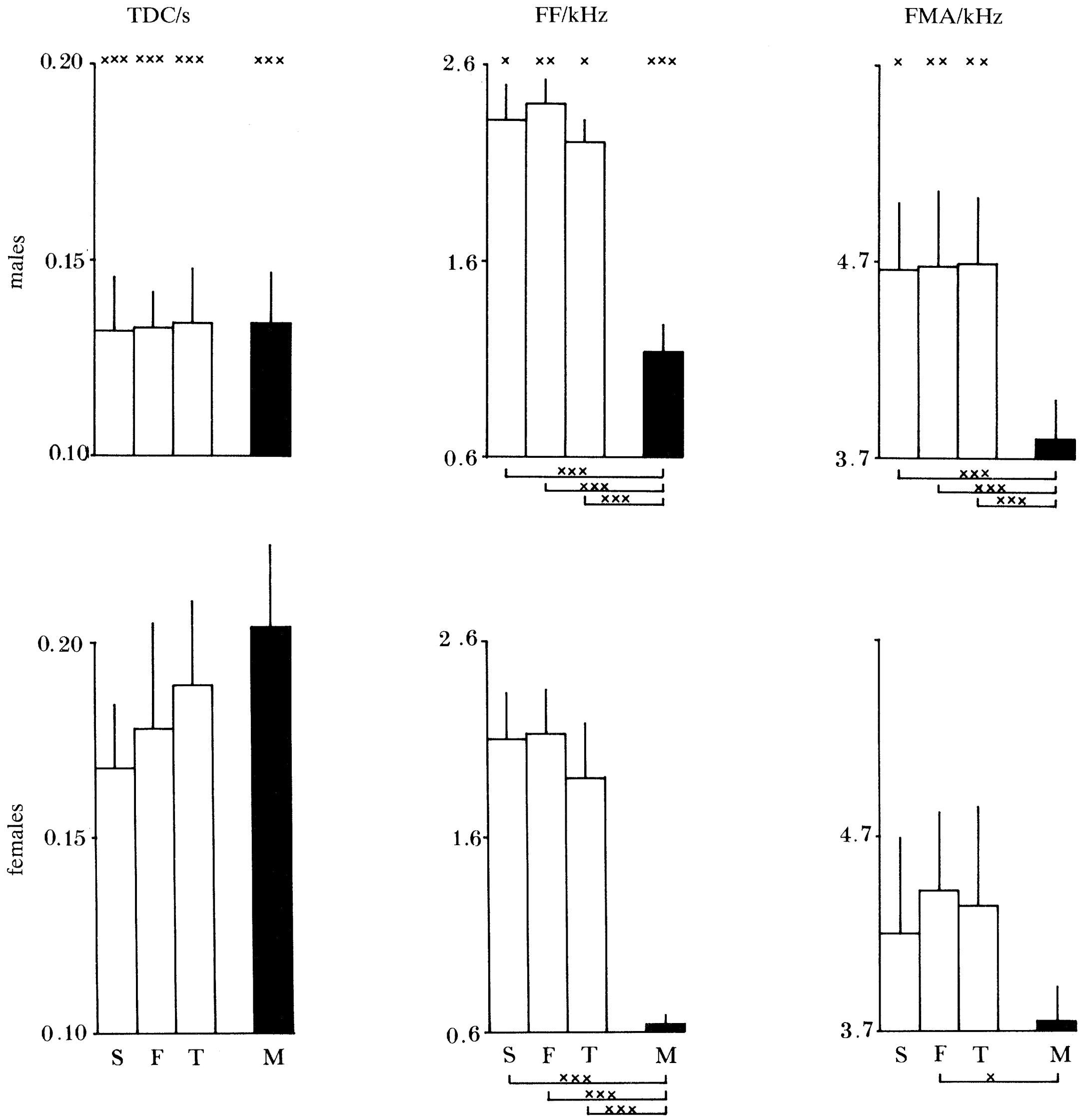

Figure 3. Histograms of distance calls of free-living *guttata* and *castanotis* (dark bars) showing mean ± s.d. in the total duration of the call (TDC, the maximum length of the loudest harmonic), the fundamental frequency (FF, the frequency of the lowest harmonic which was estimated by calculating the mean distance between visible harmonics and this value coincided with a direct measurement of the first harmonic when it was visible) and the frequency of maximum amplitude (FMA, the frequency of the darkest harmonic). Overall heterogeneity was confirmed with ANOVAS and the groups were compared with T tests. Legend as for figure 2.

it seems likely that song might serve as an important isolating mechanism between zebra finch subspecies in captivity, through female choice. Ekki Pröve and I tested the hypotheses that: (*a*) females can discriminate between the two subspecies of zebra finch male on the basis of their songs, and (*b*) females choose males of their own subspecies with longer, more complex song phrases (Clayton & Pröve 1989).

Female response to song is usually measured by playing songs to captive females implanted with oestradiol and observing their courtship displays (see, for example, Searcy & Marler (1981)). Clayton & Pröve (1989) used this method on adult birds that had had no breeding experience to test eight *guttata* and ten *castanotis* females for their response to playback of male song. Seven Bengalese finches, *Lonchura striata*, were also tested and served as a species control. All the females were normally raised by members of their own (sub)species.

Each bird was given three 24-min tests, with a 2-day interval between tests to minimize habituation. The test tapes consisted of songs recorded from normally raised from *guttata* (G), *castanotis* (C) and Bengalese finches (B) in captivity. Kroodsma (1989) has criticised many playback experiments for suffering from a lack of adequate sampling of the stimulus songs. To combat this, natural songs from 30 different males (10 *guttata*, 10 *castanotis* and 10 Bengalese finches) were used to make the test tapes. Each female was presented with nine different songs (3 *guttata*, 3 *castanotis* and 3 Bengalese finch songs), with one male of each type having a complex phrase that contained many different elements and was therefore long, one with a short, simple phrase and one that was intermediate between

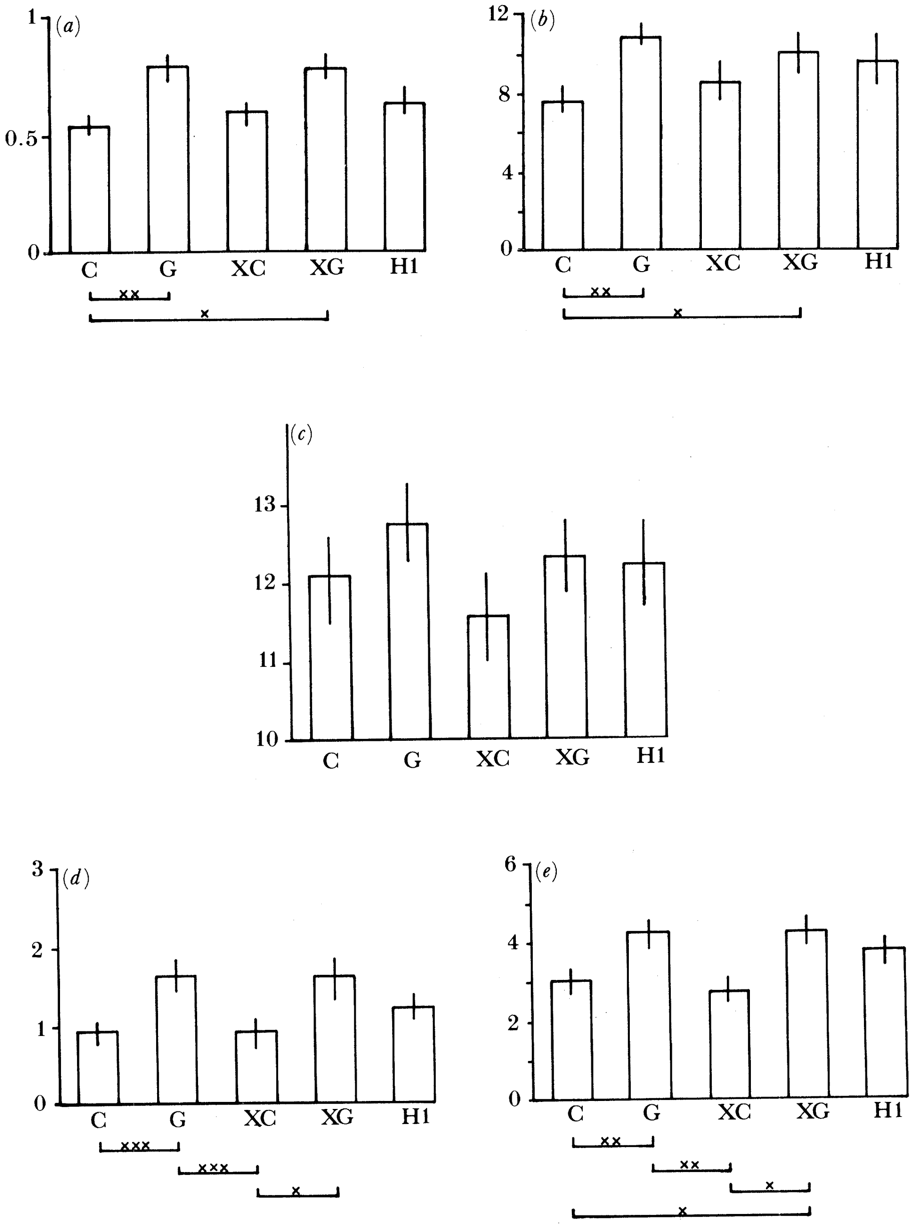

Figure 4. Histograms of mean ± s.d. in (*a*) phrase length (in seconds, in (*b*) number of elements per phrase, in (*c*) tempo (number of elements per second), in (*d*) fundamental frequency (in kHz) and in (*e*) frequency of maximum amplitude (in kHz). A total of 71 captive zebra finches were compared from 18 *castanotis* (C), 18 *guttata* (G), 12 cross-fostered *castanotis* (XC), 12 cross-fostered *guttata* (XG), and 11 hybrids (H1) from *castanotis* female and *guttata* male pairs. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ANOVA and T tests.

the two. For each test, individual females were exposed to a 4-min tape song of each type (G, C and B), chosen at random, presented in random order and each followed by intervals of 4 min silence. To control for the number of phrases heard, each female received five song phrases every 10 s during each 4 min presentation of song. We counted the number of copulation solicitation displays, in which a female crouches in a horizontal posture with the tail raised and if she is highly motivated, she vibrates the tail rapidly up and down, a movement known as tail quivering (Morris 1954). Since tail quivering does not always accompany horizontal crouching we summed the two measures.

All 25 females gave significantly stronger responses to song from conspecific males of their own subspecies than to song from males of the other subspecies or to heterospecific song (figure 5*a*), confirming that responses can be interpreted in the light of species and subspecies recognition. *Guttata* and *castanotis* significantly preferred song from males of the other subspecies to Bengalese finch song whereas Bengalese finch females did not discriminate between the songs of the two zebra finch subspecies. This reflects species differences in discrimination and the importance of the difference between *guttata* and *castanotis* songs for female zebra finches.

The conspecific male (own subspecies) with the shortest, simplest song phrase elicited significantly fewer female responses than the male with the longest, most complex song phrase and females gave an

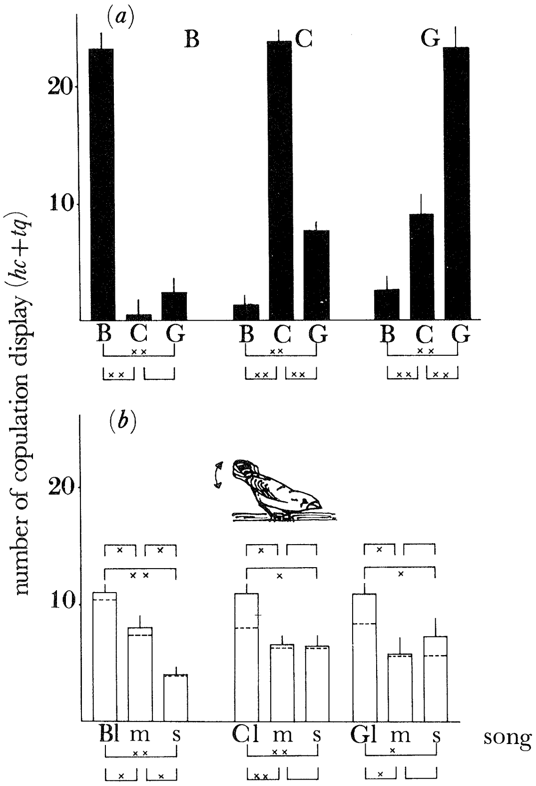

Figure 5. (*a*) Responses of 25 oestradiol-implanted females (seven Bengalese finches (B), 10 *castanotis* (C) and eight *guttata* (G) zebra finches) to playback of recorded song from three Bengalese, three *castanotis* and three *guttata* males. The mean number of copulation solicitation displays ± s.d. per 12-min song presentation for each group of females are shown. (*b*) Responses of the same 25 females to the songs of three consubspecific males that differ in the length of their song phrases. Bengalese finch males had 13 (Bl), 11 (Bm), 7 (Bs) elements per phrase (1.17 s, 1.09 s, 0.64 s); *castanotis* males had 12 (Cl), 8 (Cm), 4 (Cs) elements per phrase (0.96 s, 0.70 s, 0.41 s); *guttata* had 15 (Gl), 13 (Gm), 10 (Gs) elements per phrase (1.22 s, 0.85 s, 0.62 s). Responses are per 4 min of song presentation. Dotted lines show mean responses to 1 min, 1 min 5 s, 1 min 50 s for Bl, Bm, Bs phrases; to 1 min, 1 min 20 s, 2 min 20 s for Cl, Cm, Cs phrases; to 1 min, 1 min 26 s, 1 min 58 s for Gl, Gm, Gs phrases. The bracket above figure 5*b* refers to the dotted line responses. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, Wilcoxon tests having tested for overall heterogeneity with the Friedman ANOVA, (l, long phrase; m, medium phrase; s, short phrase.)

intermediate response to the song phrase of medium length and complexity. Figure 5*b* shows that this holds for all three groups. One problem is that exposure to a longer song phrase results in a longer exposure to the total amount of song and, therefore, the amount of stimulation of female receives. To control for this effect, Ekki Pröve and I compared the mean scores of females in each group after 1 min of the long phrase (Bl, Cl, Gl) with the mean scores after the time taken to play the equivalent amount of song for the medium and short song phrases. Although the response is not as strong, the preference for long, complex songs remains significant (figure 5*b*). Every attempt was made to hold variables other than phrase length and complexity constant but there were inevitable differences between individuals. For instance, in the *guttata* group, the male with the longest song phrase (Gl) sang more slowly than the other two males and therefore presented the females with fewer elements per unit time. This might explain why female response to different phrase lengths is not as clear in this group: the faster singing rate of the *guttata* males with the medium (Gm) and short song phrases (Gs) might compensate for their shorter phrase lengths.

Male zebra finches seldom repeat an element during a song phrase whereas male Bengalese finches do so regularly (Clayton 1987). This means that, for Bengalese finch males, the total number of elements per phrase need not equal the total number of different elements per phrase. Both the medium (Bm) and the long (Bl) phrases contained nine different elements. Females preferred the latter over the former, suggesting that the total number of elements per phrase might be more important than the number of different elements per phrase. An addition problem is that long, complex songs have a higher probability of containing elements that might be particularly stimulating. This difficulty is ameliorated at least in part by the variety of stimulus songs used.

These results show that female zebra finches and Bengalese finches can use male song alone in species–subspecies recognition and prefer males of their own subspecies that sing long, complex song phrases (Clayton & Pröve 1989). Since zebra finches learn their songs early in life (Slater *et al.* 1988) and their songs remain stable thereafter, it is unlikely that females are using song as a cue to male age. Song length and complexity might be indicative of male quality or may be a purely aesthetic trait. By producing a longer, more complex song phrase, the male is more effective in stimulating a female to perform a copulation solicitation display. This may increase his chance of mating with her or, once paired, may stimulate her reproductive activities, such as nest building and egg laying. These results support the hypothesis that sexual selection plays a role in the evolution of song complexity in some male passerines and, since zebra finches and Bengalese finches sing only one type of song, that this is not restricted to species in which males sing a large repertoire of songs.

There has been a tendency, at least by researches considering speciation in singing insects, to assume that sibling species, subspecies and geographic races that differ consistently in their songs are reproductively isolated because of these song differences, or that these are the only cues in species recognition. However, correlation need not imply causation. Other differences between the subspecies may be just as important, if not more important, in mediating assortative mating between *guttata* and *castanotis* in captivity. In the following subsection I consider other, visual cues which might be used to ensure positive assortative mating between the two subspecies of zebra finch.

(*b*) *Plumage: breast-band*

Most of the visual differences in size and plumage between *guttata* and *castanotis* are found in both males and females. However, the breast-band is an exclusively male feature and variation in its size is one of the most striking plumage differences between males of the two subspecies. Therefore, the differences in male breast-band seem to be a prime candidate as a cue to be used by females in choosing males of their own subspecies. To investigate whether breast-band size is important in mate choice and hence assortative mating between the two subspecies in captivity, male and female *guttata* and *castanotis* were tested for their sexual preference in a series of multiple mate choice tests and observations of subsequent pair formation (Clayton 1990*a*).

The mate-choice arena consisted of a circular central compartment surrounded by 10 smaller cages which are visually isolated from one another (Sonnemann & Sjölander 1977). The individual to be tested was placed in the central compartment where it could show a preference by approaching and perching in front of any of the ten birds offered as choices but could not form a pair bond (e.g. by clumping). The total time the bird spent on each perch for the 10-h test was measured on an event recorder. Their choices in the mate-choice arena were compared with observations of pair formations among the same individuals in the aviary where birds could form pairs freely. Following Butterfield (1970) and Silcox & Evans (1982), two birds were considered to be paired if they were recorded clumping on at least four separate occasions. This was confirmed when a pair was observed nest soliciting in front of the next box on at least four separate occasions and when they slept in the nestbox overnight.

To investigate how individuals respond to members of their own subspecies and the cues they might use in subspecies recognition I compared the results of a control study using *guttata* and *castanotis* of both sexes with those of a second study in which females of both subspecies were presented with *guttata* males only, half of whom had been painted to resemble *castanotis* males. I also contrasted the sexual preferences observed during pair formation in the aviary with those measured in the multiple mate-choice arena, before and after the individuals had paired.

In both the control and the painted *guttata* study none of the individuals had had previous breeding or courtship experience and the birds were unrelated and unfamiliar with one another.

Control group

Castanotis and *guttata* zebra finches (10 of each) were used, each subspecies group consisting of five females and five males. All these birds had been normally raised by members of their own subspecies in aviaries in captivity. The females were each tested three times in the multiple mate-choice area, with one female being tested each day. The ten cages housing the males that were being offered as choices were rotated 120° clockwise after all the females had been tested once, and a further 120° after the second set of tests to randomize site bias. The situation was reversed so that the 10 females were placed in cages and each male was given three sets of mate-choice test. Once the tests had been completed all 20 birds were housed in single-sex cages for 10 days. This reduced the possible order effects of testing on subsequent pair formation which was observed in a large indoor aviary over 30 days. After pair formation, the birds were housed in single-sex cages for a further 10 days, and then tested again in the mate-choice arena, following exactly the same procedure as in the previous set of tests.

Painted guttata group

Two groups, each consisting of five *guttata* and five *castanotis* females, were given the choice of 10 *guttata* males, half of whom had been painted to resemble *castanotis* males by using waterproof, non-toxic, Pelikan endorsing ink. All these birds had been normally raised by members of their own subspecies in captivity. The females and males were tested for their sexual preference in the multiple mate-choice arena. As for the control study, the birds were housed in single-sex groups after the mate choice tests for 10 days. Each group was then placed in an aviary so that subsequent pair formation could be monitored. Once pair formation was complete, the birds were rehoused in single-sex cages for a further 10 days as in the control study. Unlike the control study the birds were not retested in the mate-choice arena but were returned to the aviaries with five unfamiliar *castanotis* males. The effects on pair formation and social interactions between the competing males were observed for a further 30 days.

All the birds in the control group spent significantly more time near birds of their own subspecies in the multiple mate-choice arena, as did *guttata* females and unpainted *guttata* males in the painted *guttata* group. However, the *castanotis* females in the painted *guttata* group did not have the opportunity to court *castanotis* males: these females spent roughly equal amounts of time with painted and unpainted *guttata* males. The painted *guttata* males courted females of both subspecies.

Calculating a mean preference for each set of five individuals masks the fact that most birds preferred specific individuals of their own subspecies. To determine how many individuals a particular bird preferred, 95 % confidence intervals were calculated for each bird in each replicate and, combining the three replicates, a mean value per bird. A bird was considered to show a preference for a particular individual if the proportion of time spent with that individual lay outside the confidence interval. All the birds had one to three preferred individuals. In the control group first preference was always given to a member of their own subspecies. In the painted *guttata* group, *guttata* females also preferred unpainted males of their own subspecies, whereas six out of the 10 painted *guttata* males chose *castanotis* females as their first choice, even though they spent similar mean amounts of time with females of both subspecies. Even though *castanotis* females did not differ significantly in the amount of time spent with painted and unpainted *guttata* males, nine out of the 10 females selected painted *guttata* males as their first choice. These results are highly consistent between the three replicates, with the preferred individuals in the first mate-choice tests also being preferred in the subsequent two tests.

Observations of subsequent pair formation in the aviary showed that the birds in the control group paired with members of their own species and both sexes tended to pair with the birds that they had preferred in the previous mate choice tests, if those individuals were not already paired. I used Wilcoxon matched-pairs signed-ranks tests, comparing the proportion of time each individual spent with its eventual mate with the average proportion of time spent with other individuals of the same subspecies excluding those individuals that were already paired (control group: $z = 2.19$, $n = 9$, $p < 0.05$; $z = 2.43$, $n = 9$, $p < 0.01$; painted *guttata* group: $z = 2.72$, $n = 19$, $p < 0.01$; $z = 2.90$, $n = 19$, $p < 0.01$ for females and males in each group, respectively).

Birds spent more time with their mate in the multiple mate choice tests after pair formation than before. Of the eighteen birds that formed pairs, 13 preferred their mate in subsequent mate choice tests. The remaining three males and two females (one female died) were the last to form pairs and these pairs did not lay eggs. Four out of the five preferred the individuals they had chosen in the initial mate choice tests rather than the individual with whom they eventually paired.

In the painted *guttata* group, nine out of ten mixed pairs were formed between painted *guttata* males and *castanotis* females, nine unpainted *guttata* pairs were formed and one painted *guttata* male paired with a *guttata* female. Three out of the nine *castanotis* females that formed pairs with painted *guttata* males re-paired with *castanotis* males. These new pairs were formed on the second and third day after the *castanotis* males had been introduced. The females that had re-paired had not laid eggs whereas all the other females had begun egg-laying.

These results show that zebra finches of both subspecies prefer to mate with members of their own subspecies. Systematic studies are required to discover which cues females use in choosing a suitable mate and the relative importance of these factors during different stages of courtship. In the painted *guttata* group, *castanotis* females paired with painted *guttata* males and most of these remained paired with these males even when *castanotis* males were introduced. The *castanotis* female – *guttata* male pairings cannot be explained solely by a preference of *guttata* females for unpainted *guttata* males for two reasons. First, in the control group, and in a previous study by Böhner *et al.* (1984), members of the two subspecies did not pair up, even when the only members of the opposite sex with whom they could pair belonged to the other subspecies. Secondly, the consubspecific pairs were not formed before the mixed pairs, as would be predicted if only the *guttata* females were discriminating between painted and unpainted *guttata* males. Thus the results suggest that the difference in breast-band size is one factor which can maintain sexual isolation between the two subspecies in captivity.

The results also show that both sexes show a significant tendency to pair with their first choice in the mate choice tests, if that individual is available as a mate. Most studies of mate choice have assumed that the amount of time females spend near stimulus males in mate-choice arena is a measure of her sexual preference for those males. However, it is only recently that this measure has been validated directly: ten Cate & Mug (1984) showed a close relationship between the amount of time spent near each of the stimulus males and the amount of tail quivering (overt female sexual behaviour) to each of those males. The results presented in this subsection extend the finding that the time spent near individuals in multiple mate choice tests is an accurate indicator of sexual preference by combining the mate-choice test approach, in which the behaviour of both the tested birds and the stimulus birds are recorded, with observations of pair formations in the aviary, where there is free choice and the test conditions approximate to the natural situation in the bush. Since the birds being chosen and the test individual could interact with one another both visually and vocally during the tests it is not possible to conclude which sex chooses or, indeed, whether the final decision is mutual. This point will be discussed further in §4*b*.

(*c*) *Conclusions*

Taken together, the results presented in §3*a*, *b* show that both song and breast-band size could play a role in mate choice and subspecies discrimination, thus leading to assortative mating between the two subspecies in captivity. The next obvious step is to assess the relative importance of these cues for assortative mating. One way of doing this is to present females with two types of males, those that sing the 'right' subspecies song but possess the 'wrong' plumage and those that have the typical plumage characteristics of their own subspecies but sing the 'wrong' song, and see which type of male females prefer. By using males that had been cross-fostered to the other subspecies I hoped to determine the relative importance of visual and vocal cues, by obtaining males that had copied the song of their foster-father but resembled their own subspecies in plumage. Comparing the cross-fostered zebra finches with those that had been normally raised by members of their own subspecies allowed me to examine not only the effect of cross-fostering on song and plumage development, but also the extent to which female preferences for song, and male and female preferences for a mate, are governed by early rearing experience.

4. WHAT ARE THE EFFECTS OF CROSS-FOSTERING?

Before considering in detail the effects of cross-fostering it is worth spending some time describing the conditions under which the birds are reared because subtle, and at first sight seemingly trivial, differences in rearing regime can result in the development of quite different sexual preferences.

Each aviary contained five breeding pairs of the same type: *castanotis* pairs raising *castanotis* young (C), *guttata* pairs raising *guttata* young (G), *guttata* pairs raising *castanotis* young (XC), *castanotis* pairs raising

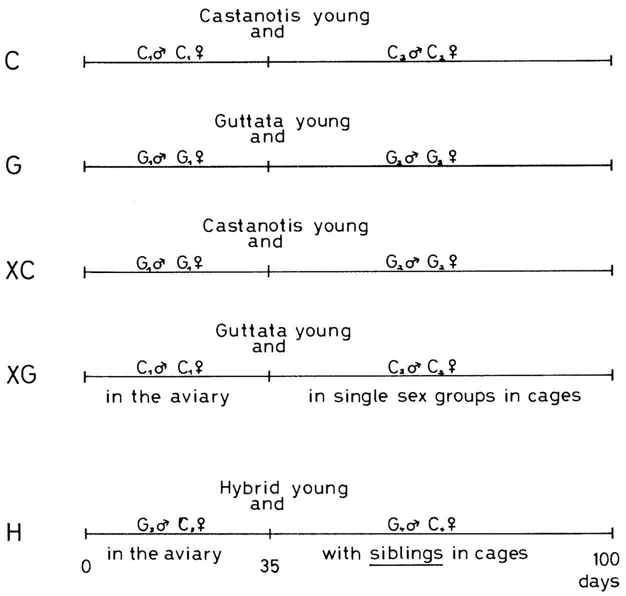

Figure 6. Rearing conditions of the zebra finches used in the cross-fostering studies. Normally raised *castanotis* (C) and *guttata* (G) young were raised by a pair of their own subspecies (C_1 and G_1, respectively) in aviaries for 35 days and then housed in single sex groups in cages until day 100 with a different adult pair of the same subspecies (C_2 and G_2, respectively). Cross-fostered *castanotis* (XC) and *guttata* (XG) young were raised by a pair of the other subspecies (G_1 and C_1, respectively) until day 35 in aviaries and then housed in single sex groups in cages with a different adult pair of the same subspecies as their foster-parents (G_2 and C_2) until day 100. Hybrid young (H1) were raised by *guttata* male and *castanotis* female pairs in aviaries until 35 days of age and then transferred to cages with both sexes of siblings and a different *guttata* male and *castanotis* female pair until day 100.

guttata young (XG) and *castanotis* female and *guttata* male pairs raising hybrid young (H). All the same-subspecies pairs raised two broods, one brood of *guttata* and one of *castanotis*. To control for the effects of egg-swapping, the eggs of these pairs were removed and replaced by a fresh clutch of consubspecific, hetero-subspecific or hybrid eggs.

The young birds remained with their foster-parents until the mean age of the brood was 35 days old (mean age of independence from the parents). From day 35 to day 100 *castanotis* and *guttata* young were removed from the aviary and housed in large cages with an adult, non-breeding tutor-pair of the same subspecies as the foster-parents. In these four groups (G, C, XG, XC) the young were housed with members of their own sex and subspecies to avoid the problem of sibling influences on the development of female song preferences (see §4*a*(ii)). To examine sibling effects the hybrid young of both sexes were housed together until day 100. For all five groups, the young from each aviary were housed separately to ensure that subjects were unfamiliar with the songs presented as test stimuli in the female preference experiment. The housing conditions are summarized in figure 6.

(*a*) ***Vocalizations: song***

To assess the extent to which the differences in songs of *guttata* and *castanotis* males are the result of rearing experiences early in life, the songs of males that had been normally raised by members of their own subspecies were compared with those of males that had been cross-fostered to the other subspecies and with those of hybrid males raised by *guttata* male and *castanotis* female pairs. The behavioural salience of these differences was then assessed by comparing the response of females implanted with oestradiol to playback of songs of *guttata* (G & XG), *castanotis* (C & XC) and hybrid (H) males (Clayton 1990*b*).

(i) *Sonagraphic differences in male song*

Songs were recorded from 71 adult zebra finches (18 *guttata*, 18 *castanotis*, 12 cross-fostered *guttata*, 12 cross-fostered *castanotis* and 11 hybrids) and analysed sonagraphically, as described in §2*c*(ii). If the differences between *guttata* and *castanotis* songs are not the result of experiences gained early in life then the songs of cross-fostered males should not differ significantly from those of normally raised males of the same subspecies (hypothesis 1). However, if the differences are learnt from adult males then the songs of cross-fostered males should be significantly different from those of males of their own subspecies and should resemble those of the foster-subspecies. As only male zebra finches sing, it also follows that the songs of hybrids raised by a *castanotis* mother and a *guttata* father should be more similar to those of *guttata*, if the differences in their songs are learnt (hypothesis 2).

A typical *castanotis* song phrase consists of about eight elements per phrase and has a duration of about 0.65 s, whereas a typical *guttata* phrase contains about eleven elements and has a duration of about 0.95 s, and that typical of a hybrid male is intermediate between the two subspecies in containing nine or ten elements per phrase, with a duration of about 0.75 s. Hybrid song elements are also intermediate in frequency, being lower in pitch than those of *guttata* but higher than those of *castanotis*.

Figure 4 summarizes the main differences between the five groups, which were compared and tested for their significance by using one way analyses of variance (ANOVA). There were no significant differences in tempo (number of elements per second), although *guttata* songs were slightly faster than *castanotis* ones. For phrase length, number of elements per phrase, frequency of maximum amplitude and fundamental frequency of the song elements significant differences were found, the largest differences being between *castanotis* and *guttata* songs, with those of hybrids having intermediate values. The songs of cross-fostered *castanotis* were slightly longer than those of normally raised *castanotis*, with more elements per phrase, whereas the songs of cross-fostered *guttata* were slightly shorter than those of normally raised *guttata*, with fewer elements per phrase. In neither case did the difference attain statistical significance and, for the frequency measures, the songs of normally raised and cross-fostered males of each subspecies were remarkably similar.

Thus cross-fostered males resemble males of their own subspecies in these macrostructural features of song which distinguish the two subspecies' songs. This, together with the result that the songs of hybrid males are intermediate between those of the two subspecies, supports hypothesis 1: the results suggest that rearing experience has little, if any, effect on the development of the macrostructural differences between the songs of the two subspecies. However, I do not wish to imply that learning does not influence song development. Indeed, comparisons of these results, and those of males raised by Bengalese finch foster-parents (Clayton 1989*b*), with the songs of males that have been raised by the mother alone in absence of the song tutor (Eales 1987), shows that some learning is involved and that exposure to any male appears to be sufficient for a young male to organise his song elements into phrases (c.f. Lickliter & Gottlieb 1988). Learning is probably most crucial at the microstructural level: young male zebra finches learn individual song elements comprising the song phrase from adult singing males (review by Slater *et al.* (1988)). With the exception of the frequency differences, no consistent differences in the fine structure of *guttata* and *castanotis* elements have been found so far.

(ii) *Female response to differences in song*

Given the differences between the songs of *guttata*, *castanotis* and hybrid males it is pertinent to ask whether females use these macrostructural differences in subspecies recognition and to what extent these preferences are learnt. If female song preferences are based on similarity to the father's song then cross-fostered females should prefer the songs of males of the foster-subspecies (hypothesis 1). Females might develop preferences for the songs of their brothers and/or other young males with whom they were housed (hypothesis 2). As young males do not develop their adult song completely until about 90 days of age, a difference between the two hypotheses would be seen in the hybrid females. These females should prefer *guttata* males if hypothesis 1 is correct but they should prefer hybrid males if hypothesis 2 is correct. Thirdly, females might learn song performances on the basis of both the foster-father's and brother's (and/or other young males with whom they were housed) songs (hypothesis 3). A fourth possibility is that female song preferences are not learnt. In this case, females should prefer the songs of males of their own subspecies, irrespective of the subspecies of the foster-parents (hypothesis 4). Alternatively, females might not discriminate between song types on the basis of the subspecies of the singer, but simply prefer longer songs (hypothesis 5). If the latter is correct then females should prefer the songs of *guttata*, cross-fostered *guttata* and hybrid males.

Seventy one females (16 *guttata*, 19 *castanotis*, 12 cross-fostered *guttata*, 13 cross-fostered *castanotis* and 11 hybrids) were implanted with oestradiol and their response to playback of male song was scored by observing the number of copulation solicitation displays (see §3*a*), the total duration of the response in *s*. and the latency to respond in *s*.

Each responsive female was given ten 5-min tests consisting of 30 s silence preceding and following a 4-min song bout from one male. To control for the number of song phrases heard, a female was exposed to five song phrases every 10 s during each 4 min of song. Natural songs from fifty different males were used to make the test tapes. Ten males were randomly chosen from each of the five groups (G, C, XG, XC, H). The song phrases were selected at random for use on the test tapes. Individual females were scored for their response to ten test tapes from unfamiliar males, two from each group, presented in random order and with two days interval between each test to avoid habituation. For each female, mean response scores were taken to the two *guttata*, *castanotis*, cross-fostered *guttata*, cross-fostered *castanotis* and hybrid songs.

Of the 71 females tested, 54 were responsive to song playback following oestradiol implantation (17 non-responsing females were evenly distributed across the five groups). Female scores to the different songs were compared and tested for their significance by using the Friedman one way analysis or variance and corresponding Wilcoxon tests, according to the WISTAT Statistical Package for multiple comparisons between matched groups ($p < 0.002$ for all comparisons except latency in group XC).

Figure 7 shows histograms of the mean number of copulation displays, the mean total duration of response and the mean latency to respond for each song type for the five groups of females. *Castanotis* females (C) and *guttata* females raised by *castanotis* parents (XG) preferred *castanotis* and cross-fostered *castanotis* songs and did not discriminate significantly between the two song types, although the scores to cross-fostered *castanotis* were slightly greater. *Guttata* females (G) and cross-fostered *castanotis* females (XC) preferred *guttata* and cross-fostered *guttata* songs but the response scores were slightly, but not significantly, greater to *guttata* song. Hybrid females preferred *guttata*, cross-fostered *guttata* and hybrid song types although the latency score to cross-fostered *guttata* song did not reach statistical significance).

These results show that female zebra finches prefer the songs of their foster-father's subspecies, irrespective of whether the songs were from males that had been cross-fostered or normally raised. That females do not seem to discriminate significantly between the songs of normally raised and cross-fostered males of the same subspecies adds further support to the sonagraphic similarities between the song types (C & XC; G & XG) reported above. However, although the scores to these song types are similar, there is a slight preference for the song of cross-fostered over normally raised *castanotis* and for the song of normally raised over cross-fostered *guttata*. For both comparisons, females show a preference for those songs that contain the greatest number of different elements (complexity) and the longest song phrase, confirming the results described in §2*a* (Clayton & Pröve 1989).

Comparisons of female preferences across the five groups suggest that song preferences are learnt. In the two cross-fostered groups (XG & XC) females prefer songs from males of the same species as their foster-parents whereas normally raised females (C & G) prefer the songs of males of their own subspecies. This comparison demonstrates the importance of paternal influence on the development of female song pref-

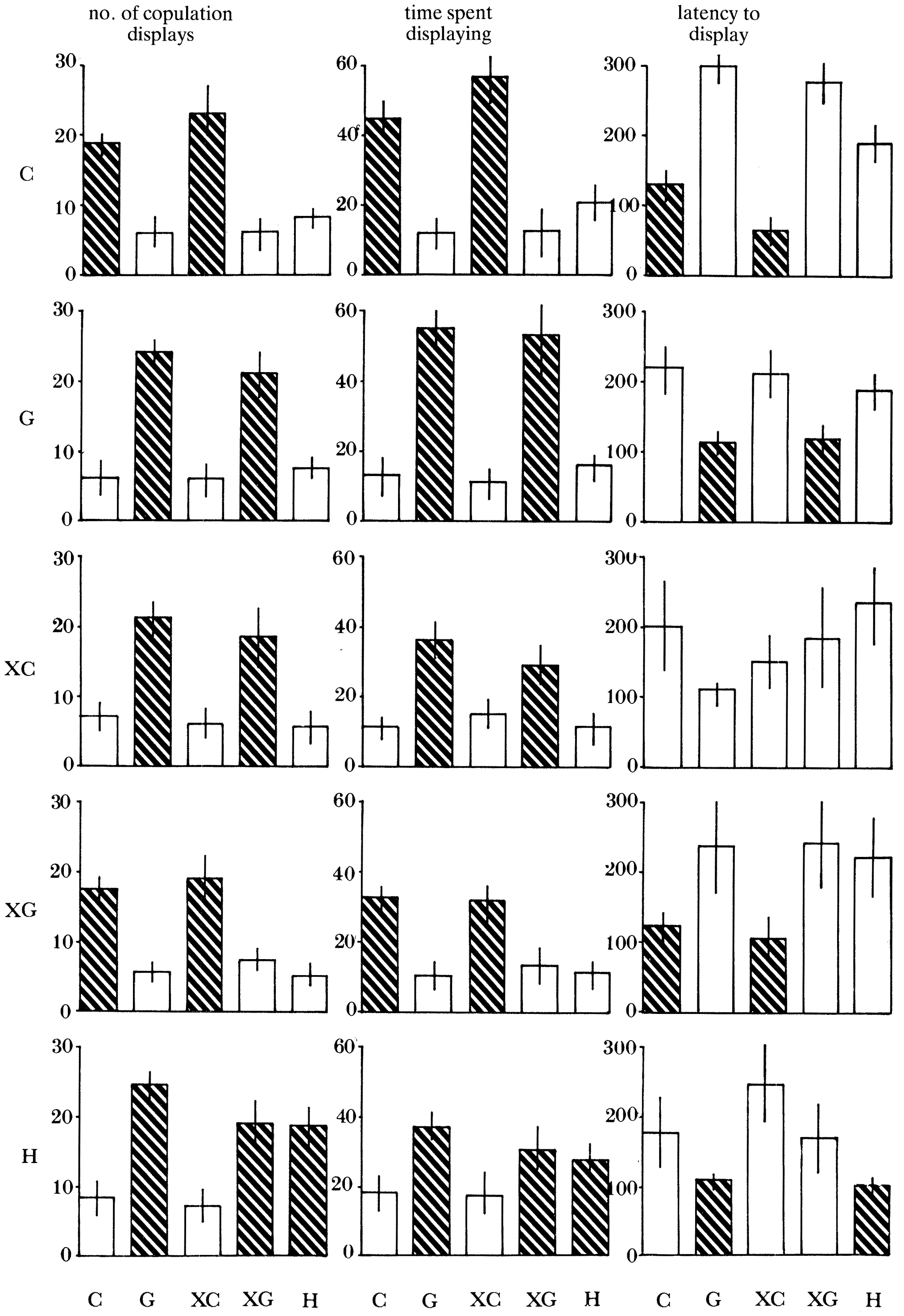

Figure 7. Responses of 54 oestradiol-implanted females (12 C, 12 G, 8 XC, 10 XG, 12 H1) to playback of recorded songs of two C, G, XC, XG and H1 songs from a sample of 50 different songs. The mean number of copulation solicitation displays ± s.d., the total duration of the copulation solicitation display ± s.d., and the latency to respond ± s.d., were calculated per 4-min song presentation for each female group. Stippled bars show female preference, i.e. females showed significantly stronger responses to song types shown by stippled bars ($p < 0.05$ in all cases, $p < 0.001$ in most cases except for latency scores, Friedman ANOVA and Wilcoxon tests). Legend as in figure 4.

erences and suggests that the learnt preference is not restricted to the father's song but is generalized to the songs of other males of the same subspecies. In these four groups females were unlikely to be influenced by their brothers and other young males because the two sexes were housed separately after Day 35, long before the young males sing their adult songs. However, in the hybrid group (H), the females were housed with their brothers until Day 100, so that they were exposed to the songs of their brothers and other young males in the aviary for at least the last 10 days. Hybrid females developed sexual preferences for *guttata*, cross-fostered *guttata* and hybrid male songs. One explanation for this is that sibling influences are important for the development of female song preferences. These results support hypothesis 3, that female song preferences are learnt from the father and from brothers and other young male aviary-mates. However, since the songs of *guttata*, cross-fostered *guttata* and hybrid males are longer than those of *castanotis* and cross-fostered *castanotis* males, the results are also in line with hypothesis 5, namely that females prefer longer songs, irrespective of the subspecies of the singer. Interestingly, hybrid females respond most to the songs of *guttata* males and these are longer than those of cross-fostered *guttata* and hybrid males, although the difference in response did not reach significance.

(iii) *Implications*

Both sets of results suggest that the songs of normally raised and cross-fostered males within each subspecies are remarkably similar in microstructure. This shows the importance of combining sonagraphic analysis with female response to playback and shows that the similarities and differences in song parameters measured appear to be the same as those used by female zebra finches in subspecies recognitions.

The similarity between normally raised and cross-fostered males' songs, and the fact that hybrid males' songs are intermediate between the two subspecies' songs, indicates that these macrostructural differences in the songs of *guttata* and *castanotis* are largely unresponsive to learning. These results necessarily meant that I was unable to assess the relative importance of visual and vocal cues by using cross-fostered birds as originally planned since males were not copying those features of their foster-father's song that females appear to use in discrimination between the two subspecies.

(*b*) *Visual cues: size and plumage*

One of the most interesting points described in the previous subsection is that female zebra finches do not discriminate between normally raised and cross-fostered males of their own subspecies on the basis of song. This raises the question of whether females can discriminate between normally raised and cross-fostered males and, if so, which cures they might use. Given that male *guttata* and *castanotis* also differ in size and in their plummage, females might rely on either or both of these cues.

In this subsection I consider whether females prefer normally raised males of their own subspecies over those that have been cross-fostered. In order to assess which cues females might use it is necessary to consider whether early rearing experience can affect the subspecies differences in size and plumage. Having compared the size and plumage of normally raised *guttata* and *castanotis* with that of cross-fostered and hybrid zebra finches, I assess the behavioural significance of these results by comparing female preferences for normally raised and cross-fostered males of both subspecies in multiple mate choice tests.

Normally raised *guttata* (G; 10 males, 10 females) and *castanotis* (C; 10 males, 10 females) were compared with cross-fostered *guttata* (XG; 10 males, 10 females) and cross-fostered *castanotis* (XC; 10 males, 10 females) and with both hybrids raised by *castanotis* female – *guttata* male pairs (H1; 10 males, 11 females) and hybrids raised by *guttata* female – *castanotis* male pairs (H2; 3 males, 3 females).

(i) *Differences in size*

Figure 8 shows that cross-fostered birds do not differ significantly from normally raised males of the same subspecies in size. For both sexes, *castanotis* were significantly larger than *guttata*, with both types of hybrid being intermediate between the two subspecies. No significant differences between the two types of hybrids were found.

(ii) *Plumage differences*

For the six groups, the bill colour of both sexes and the male breast-band size were measured. For both bill colour and male breast-band size, there were significant differences between *castanotis*, *guttata* and the hybrids ($p < 0.05$ in all male comparisons, having confirmed for overall heterogeneity in bill colour and breast-band size with Kruskal Wallis H tests; for females for Kruskal Wallis H test was not significant). However, the two types of hybrids did not differ significantly from each other and the cross-fostered birds resembled normally raised birds of their own subspecies.

(iii) *Female preference*

The result that cross-fostered zebra finches resemble normally raised members of their own subspecies, with hybrids (H1 & H2) being intermediate in size and plumage between *guttata* and *castanotis*, suggests that females are unlikely to be able to discriminate between cross-fostered and normally raised male zebra finches of the same subspecies on the basis of the size or plumage that were measured. However, these males might differ in their behaviour as a result of differences between them in rearing experiences: females might use such behavioural cues to discriminate between normally raised and cross-fostered males.

To test this possibility, the preferences of ten female *guttata* and ten female *castanotis* for normally raised and cross-fostered males of both subspecies were tested in a multiple mate-choice arena. As described in §2*b*, the mate-choice arena consisted of a circular central compartment surrounded by 10 smaller cages which were visually isolated from one another.

Each female was presented with eight males, two of each type (i.e. 2 G, C, 2 XG & 2 XC). To increase the sample size of the males being used in the tests, two males of each type were selected at random for each test from a stock of 10 males of each type. The female to be tested was placed in the central compartment and eight males were placed in the small, surrounding cages, with *castanotis* (C or XC) being placed in positions 1, 3, 5 and 9 and *guttata* (G or XG) in positions 2, 4, 6 and 8. The remaining two cages, in positions 7 and 10, were empty. The cages were rotated one position (i.e. 36° clockwise) after each individual had been tested to randomize site bias.

The female could show a preference by approaching or perching in front of any of the eight birds (and two

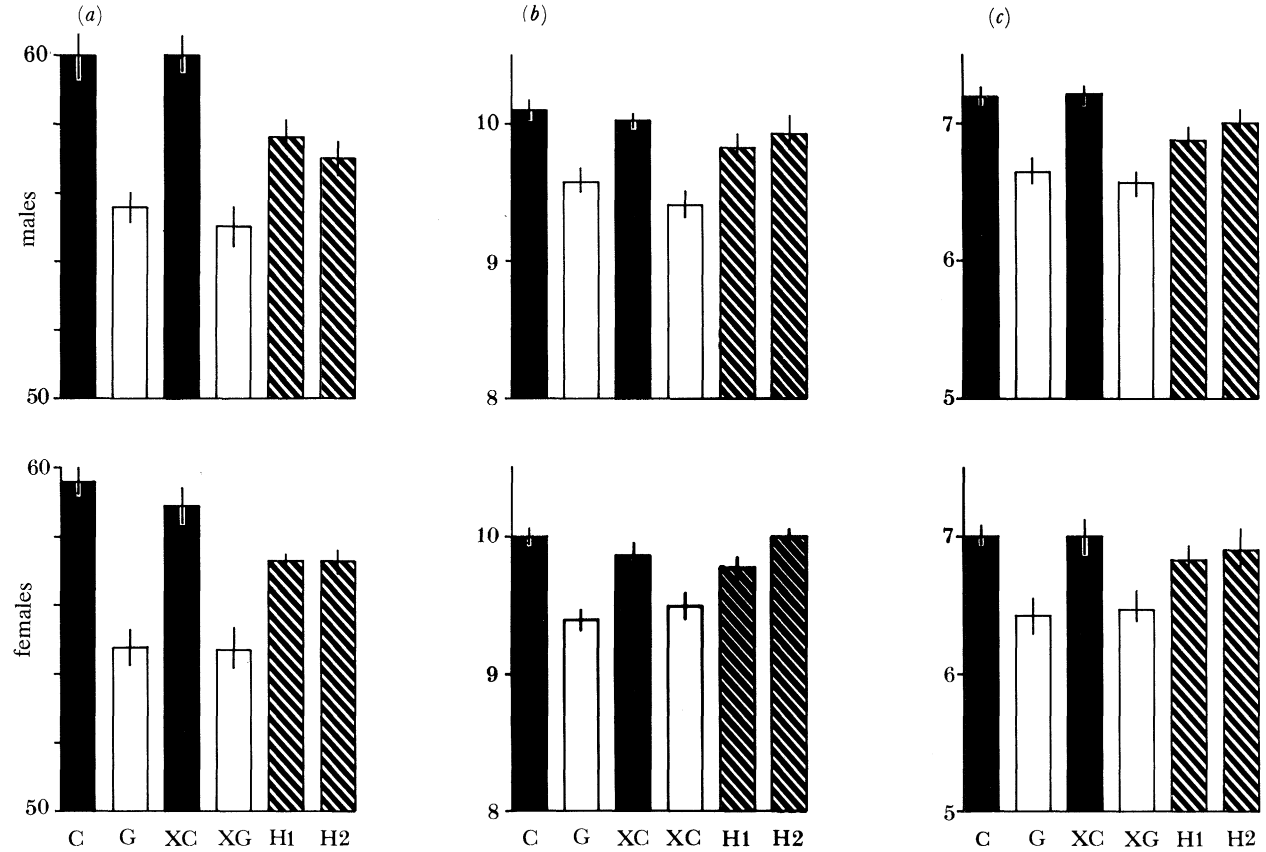

Figure 8. Histograms comparing C (10 males, 10 females), G (10 males, 10 females), XC (10 males, 10 females), XG (10 males, 10 females), H1 (10 males, 11 females), and H2 hybrids from *castanotis* male and *guttata* female pairs (3 males, 3 females). For each group mean ± s.d. in (*a*) wing length, (*b*) bill length and (*c*) bill depth are shown. Overall, heterogeneity was confirmed per group and sex for each measure by using oneway ANOVAS and the groups were compared with T tests. Filled bars show that *castanotis* were significantly larger than *guttata* (open bars), irrespective of rearing experience ($p < 0.001$ for all six comparisons) and hybrids H1 and H2 (stippled bars) were significantly different from both subspecies, being intermediate between the two in size ($p < 0.05$ for all comparisons).

empty cages) offered as choices and, for each 10-h test, the total time the female spent on each perch was measured on an event recorder. Given the highly significant correlation between preference scores across successive mate choice tests found in §2*b* (Clayton 1990*a*), each female was tested only once in this group.

For each female, the total amount of time spent with normally raised males, as a proportion of the total time spent with both normally raised and cross-fostered males of their own and of the other subspecies was calculated. The two sets of scores were compared for *guttata* and for *castanotis* females using Wilcoxon matched pairs sign ranks test: females of both subspecies preferred normally raised males of their own subspecies over those that has been cross-fostered whereas they spent approximately equal amounts of time with normally raised and cross-fostered members of the other subspecies (*guttata* $T = 53$, $n = 10$, $p < 0.001$; *castanotis* $T = 54$, $n = 10$, $p < 0.01$).

This result indicates that female *guttata* and *castanotis* can discriminate between normally raised and cross-fostered males of their own subspecies and that they prefer those males of their own subspecies that have been normally raised. Since females do not show a preference when presented with male song in the absence of visual cues (§4*a*), and no significant difference in size or plumage could be detected (§4*b*) (i), (ii)), this result suggests that females discriminate between normally raised and cross-fostered males of their own subspecies either on the basis of behavioural differences between the two or because the combination of visual and vocal cues improves their powers of discrimination.

In the following subsection the behavioural cues are considered in more detail by describing an experiment in which the influence of early rearing experience on assortative mating was assessed by comparing the sexual preferences of normally raised and cross-fostered males and females of the two subspecies (Clayton 1990*a*).

(*c*) *Assortative mating*

Following the procedure described above and in detail in §3*b*, three groups of cross-fostered and normally raised *guttata* and *castanotis* of both sexes were tested for their sexual preference in mate choice tests and in pair formation in the aviary. All the individuals were unrelated and unfamiliar with each other.

In the multiple mate choice tests, an individual could show a preference for perching near a certain individual but physical contact was not possible. Two sets of multiple mate-choice test were given. In the first set, one way mirrors were placed between each of the small outside cages and the central compartment so that the stimulus birds in the outside cages could not see the bird being tested but the bird in the central compartment could see the stimulus birds. Males and then females were tested, so that the females offered as choices for the males were subsequently given the choice of which of the males that had previously served as subjects they preferred. One bird was tested each day until all the birds had been tested once. When all the birds had been tested, the procedure was repeated in two-way tests, in which the mirrors were replaced by wire mesh screens that allowed visual interaction between the test individual and the stimulus birds. As described in the previous subsection, two stimulus birds of each type (G, C, XG, XC) were presented, with *castanotis* (C or XC) being placed in positions 1, 3, 5 and 9 and *guttata* (G or XG) in positions 2, 4, 6 and 8. The remaining two cages, in positions 7 and 10, were empty. The cages were rotated 36° clockwise after each individual had been tested to randomize site bias.

Choices in the one-way and two-way tests were compared with each other and with observations of pair formation in indoor aviaries, where the same birds could form pairs freely. As described in §3*b*, for the pair formation tests, birds were placed in indoor aviaries, nest boxes were added and daily observations were made to determine when the birds paired. Birds were said to have formed a pair if they were recorded clumping on at least four separate occasions, This was confirmed when a pair was observed nest soliciting in the vicinity of the nest box or when they slept in the nest box overnight.

(i) *Cross-fostered male and female group*

Two sets, each consisting of five cross-fostered *guttata* (XG) males, five cross-fostered *castanotis* (XC) males, five cross-fostered *guttata* (XG) females and five cross-fostered *castanotis* (XC) females were measured in a one-way and a two-way test in the multiple mate-choice arena. Once the mate choice tests had been completed, each group of 20 birds was rehoused in single-sex cages for 10 days. This reduced the possible order effects of testing on subsequent pair formation which was observed over 30 days.

(ii) *Cross-fostered male group*

Two sets of five cross-fostered *guttata* (XG) males, five cross-fostered *castanotis* (XC) males, five *guttata* (G) females and five *castanotis* (C) females were tested for their sexual preferences. The procedural details are identical to those described above.

(iii) *Cross-fostered female group*

The sexual preference of each of two sets of five *guttata* (G) males, five *castanotis* (C) males, five cross-fostered *guttata* (XG) females and five cross-fostered *castanotis* (XC) females were tested for their sexual preferences, following the above described procedure.

The number of individuals in each study that preferred their own or the other subspecies, and the number of individuals that showed a preference for both subspecies, is shown in table I. An individual was said to show a preference for a particular bird if the proportion of time spent with that bird lay outside the 95% confidence limit of the time spent with each of the stimulus birds in the multiple-choice tests. Cross-fostered birds differed significantly from normally raised birds in preferring members of the foster subspecies in preference to those of their own subspecies ($\chi^2 = 87.71$, df = 10, $p < 0.001$; χ^2 tests, df = 1, $p < 0.001$ for all comparisons between normally raised and cross-fostered groups). A further difference between normally raised and cross-fostered birds was that some of the cross-fostered birds showed a preference for members of both subspecies (Fisher exact tests $p < 0.05$ in the cross-fostered female group; $p < 0.001$ in the cross-fostered male group). The number of cross-fostered males that showed a preference for both subspecies was significantly greater in the cross-fostered male group than in the cross-fostered male and female group (Fisher exact test, $p < 0.01$) whereas the difference between cross-fostered females in the cross-fostered female group and the cross-fostered male and female group did not attain significant (Fisher exact test, $p < 0.05$).

Table 1. *Subspecies preferences in the mate-choice tests described in Section 4c*

	no. birds that show a subspecies preference					
	in the one-way test for			in the two-way test for		
group	own	other	both	own	other	both
XX						
XCm	2	5	3	1	7	2
XGm	2	6	2	0	9	1
XCf	1	7	2	1	8	1
XGf	1	6	3	1	7	2
XM						
XCm	0	3	7	5	0	5
XGm	1	5	4	1	1	8
Cf	10	0	0	9	0	1
Gf	10	0	0	10	0	0
XF						
Cm	10	0	0	4	2	4
Gm	10	0	0	5	1	4
XCf	3	4	3	5	4	1
XGf	0	7	3	5	5	0

Abbreviations used: XX, cross-fostered male & female group; XM, cross-fostered male group; XF, cross-fostered female group.
C, *castanotis*; G, *guttata*; XC, cross-fostered *castanotis*; XG, cross-fostered *guttata*; m, male; f, female.

Observations of subsequent pair formation showed that birds in the cross-fostered male and female group formed mixed pairs with members of the foster-subspecies (one cross-fostered *castanotis* male died, leaving one cross-fostered *guttata* female unpaired). In contrast, only three mixed pairs and 13 consubspecific pairs were formed in the cross-fostered female group (in one set, one cross-fostered *guttata* female died, and two *guttata* males and one cross-fostered *guttata* female

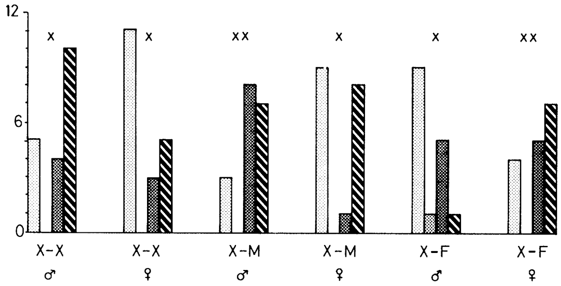

Figure 9. Number of birds showing a preference for the mate in both the one-way and two-way tests (▦), in the one-way test only (▦), in the two-way test only (▦), and in neither test (▨) were compared with the McNemar change test. XX cross-fostered male & female group (19 males, 19 females); XM cross-fostered male group (18 males, 18 females); XF cross-fostered female group (16 males, 16 females).

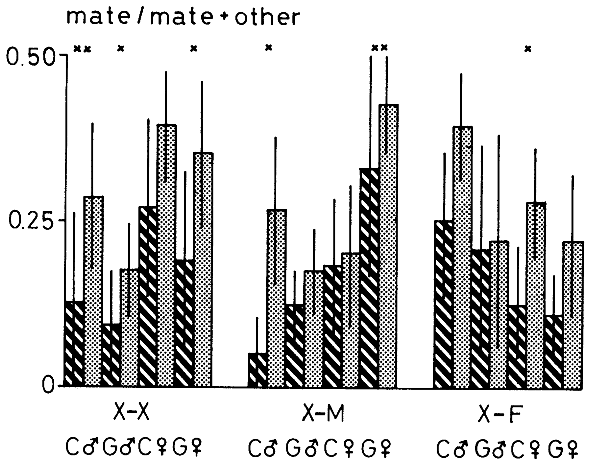

Figure 10. Mean ± s.d. of the time males (M) and females (F) spent near the future mate, as a proportion of time spent with all ten choice birds, in the one-way (▩) and two-way tests (▦) were compared with the Wilcoxon test. XX (19 males, 19 females); XM (18 males, 18 females); XF (16 males, 16 females). Legend as for figure 9.

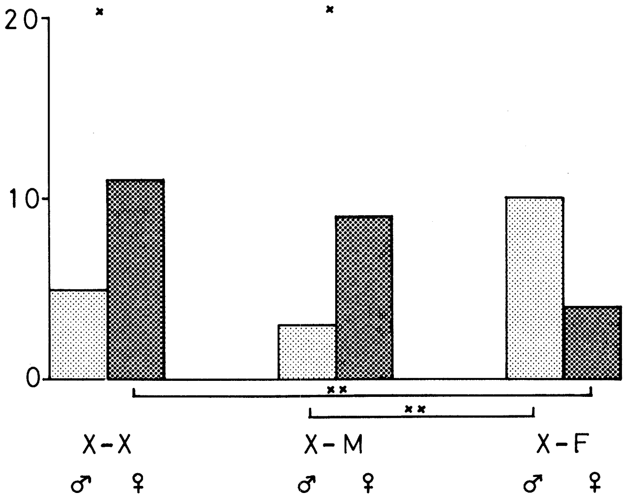

Figure 11. Number of males (▦) and females (▦) that prefer the future mate in the one-way multiple mate-choice test were compared (asterisks above) and each sex was compared between groups (asterisks in brackets below) with χ^2 tests, having tested for overall heterogeneity ($\chi^2 = 4.67$, df = 7, $p < 0.05$). XX (19 males, 19 females); XM (18 males, 18 females); XF (16 males, 16 females). Legend as for figure 9.

did not pair; in the other set, two birds died, leaving one unpaired cross-fostered *guttata* female and one unpaired *guttata* male). In the cross-fostered male group all the birds paired with members of their own subspecies (in each set, one bird died leaving one unpaired bird). The results show that when both sexes are cross-fostered, mixed subspecies pairs are formed, whereas if only one sex is cross-fostered, the majority of birds (31 out of 34) pair with members of their own subspecies, irrespective of their initial preference. This is particularly striking in the cross-fostered male study where the females were normally raised. This suggests that the ultimate choice of subspecies of partner lies with normally raised birds, particularly if they are female.

To examine whether interaction between the test individual and the birds being given as choices influences mate choice, the number of males and females in each study that preferred the future mate or a non-mate in the one-way and two-way multiple mate-choice tests was calculated. The results are shown in figure 9. McNemar change tests show that the results of the one-way and two-way tests are significantly different for both sexes in all three groups. Figure 10 shows that in all three groups birds spent more time with their subsequent mate, as a proportion of the total time spent with all stimulus birds, in the two-way tests than in the one-way tests. Comparisons of the one-way and two-way multiple mate-choice tests show how the initial preferences for a particular subspecies can differ from that of the final choice of partner, reflecting the importance of behavioural interactions between the individual being tested and the stimulus birds for mate choice and assortative mating.

χ^2 tests of the number of individuals that preferred the future mate in the one-way tests versus the number that did not shows that normally raised birds are more likely to become paired with their choice of partner than cross-fostered birds and that, when both sexes are cross-fostered, then females do the choosing (figure 11).

The results of the cross-fostered male and female study suggest that behavioural differences in subspecies preference, which develop as a result of sexual imprinting, potentially could be an important factor in maintaining sexual isolation between *guttata* and *castanotis* in captivity. The result that zebra finches of both sexes initially preferred, and subsequently paired with, members of the foster-subspecies is in line with those of other studies in both the laboratory (Immelmann *et al.* 1978) and in the field (review by Cooke & Davies (1983)). However, the results of the other two groups show that sexual imprinting on the parents is not the only factor involved. Despite their initial preferences in the one-way test, all the males in the cross-fostered male group, and most of the females in the cross-fostered female group, paired with members of their own subspecies.

Another factor which might influence the birds' sexual preferences is early experience with siblings. The cross-fostered birds may have learned some characteristics of their own subspecies in addition to those of their foster-subspecies because these birds encountered their siblings and age-mates of their own subspecies in the aviary before independence and were housed in single-sex groups with consubspecifics after day 35. This means that the normally raised birds only experienced their own subspecies early in life whereas the cross-fostered birds were exposed to both subspecies. This difference in experience might explain why the cross-fostered birds tended to 'dither' (i.e. show a preference for both subspecies) whereas the normally raised birds did not.

There are good functional reasons for expecting females to be the 'choosy' sex (Trivers 1972). Comparisons between the groups, subspecies and sexes show that in both the cross-fostered male and female group and in the cross-fostered male group more females had the ultimate choice of partner than males. However, in the cross-fostered female group more males chose than females. This suggests that when both sexes are cross-fostered, and presumably when both sexes are normally raised by their own subspecies, female choice is the limiting factor. However, when one sex is cross-fostered and the other is normally raised, the latter choose, irrespective of sex. Perhaps the normally raised birds outcompete their cross-fostered counterparts in some way, by showing more persistence in their courtship, for example, and therefore are chosen, regardless of initial preference (see, for example, Brodsky *et al.* (1988)). Whatever the reason, these results show that normally raised birds are more likely to pair with their preferred choices than cross-fostered birds. The results of the cross-fostered female group and cross-fostered male group suggest that cross-fostered individuals tend to pair with members of their own subspecies, indicating that the likelihood of pairing with the 'wrong' subspecies is minimised when cross-fostered individuals interact with members of their own subspecies. Potentially, this would provide an adaptive mechanism for maintaining behavioural isolation between the two subspecies.

5. LINKING THE DEVELOPMENT AND FUNCTION OF BEHAVIOUR

Mayr (1970) suggested that widely separated allopatric populations may develop pre-mating isolation mechanisms, either by chance or as an incidental result of adaptive changes in other characters in different selective regimes (see also discussion in Endler (1989)). In support of Mayr's hypothesis, Böhner *et al.* (1984) and Clayton (1990*a*, §3*b*) have shown assortative mating between two geographically isolated zebra finch subspecies in captivity. Mayr (1970) also claimed that 'behavioural differences between geographic races do occur but it is rarely known to what extent they have the potential to effect reproductive isolation'. The results in §4*c* (Clayton 1990*c*) suggest that behavioural differences in subspecies preference, which develop through sexual imprinting on the foster-parents, could potentially be an important factor in maintaining sexual isolation between *guttata* and *castanotis* in captivity.

In view of the potential role of song and plumage for mate choice it is crucial that females use cues which reliably indicate to which subspecies a particular male belongs. Recent work by Balaban (1988*a*, *b*) suggests that female swamp sparrows, *Melospiza georgiana*, prefer males that sing their own dialect and that these song preferences are determined by the female's population of origin. It seems likely that zebra finches use different rules: females learn to prefer males with certain types of song but the features of song that females use to recognize their own subspecies are not learnt. In both males and females, mate preferences are learnt. As is the case with song features, the plumage cues used in subspecies recognition are also those that are not affected by rearing experience.

Although stereotypy is important for subspecies discrimination, it is generally thought that variety is advantageous for mate choice and territorial defense between consubspecifics. In zebra finches, learnt microstructural differences in the songs of individuals (e.g. similarity to father's song, see, for example, Clayton (1987)) are thought to influence which male members of their own subspecies females prefer. Selection for song learning may have arisen because errors resulting from the song learning process allow rapid cultural change in song and that such change may confer some advantage to an individual.

Given the conflict between stereotypy and variety one question that remains unanswered is the extent to which the cues used for assortative mating between subspecies are also those used for mate choice within the subspecies. For zebra finches, macrostructural features of song appear to be used for subspecies discrimination and learnt microstructural differences for mate choice between individual members of the same subspecies. Section 4*b* (Clayton 1990*b*) suggests that the learnt behavioural differences between zebra finch males of the same subspecies that have been either normally raised or cross-fostered to the other subspecies can also be employed. For visual features, it remains to be seen whether females prefer members of their own subspecies on the basis of breast-band size or whether other cues, e.g. bill colour or size, are of more importance.

I dedicate this article to the late Klaus Immelmann, without whose inspiration this project would not have been possible. Tragically, he died just days before my arrival as a NATO post-doctoral fellow (funded by the SERC) in his department at the University of Bielefeld, West Germany. I thank all my friends in the Ethology Department for being so helpful and kind, and for making my stay there so enjoyable. Hans-Joachim Bischof deserves special mention for his seemingly unending source of support and patience, and for always finding time to discuss my ideas, no matter how busy he was. I am very grateful to Ekki Pröve, with whom I collaborated on the song discrimination project described in §3*a*. He taught me how to implant females with oestradiol and carried out most of the implantations himself. The field work described in §2 was supported by travel grants from ASAB, BOU, the Percy Sladden Memorial Fund and the SERC, to whom I am very grateful. This field work was carried out with the help of Dave Hodson (in Indonesia) and Richard Zann (in Australia) and I thank both of them for their help in the field. Peter Slater kindly allowed me to use his sound recording equipment in the field and to use his sonagraph for analysis on my return. I thank him for being a great host and for the numerous discussions about zebra finches. I am also indebted to Edda Geissler and Mike Amphlett for preparing the figures. Last, but not least, I thank Hans-Joachim Bischof, Emma Brindley, Marc Cellier, Frank Cezilly, John Krebs, Pete McGregor, Mike Shepherd and Peter Slater for reading drafts of the manuscript and shaping it into its present, considerably improved form.

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