

The effect of early exposure to the opposite sex on mate choice in White Leghorn chickens

Marty L. Leonard^{*}, Liana Zanette¹, Michael Clinchy²

Centre for Food and Animal Research, Central Experimental Farm, Ottawa, Ont. K1A 0C6, Canada

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Abstract

Exposure to the opposite sex during rearing affects both behaviour and mating success in White Leghorn chickens. In particular, pairs of males and females with similar experience (i.e. both reared either with or without the opposite sex) have higher mating success than pairs with different early experience. Given these results, we predicted that females should prefer to mate with compatible males reared under conditions similar to their own. During mate choice trials, females chose males with similar rearing conditions when they could see, but not interact directly with the males. However, when females could interact with males, their choices were random with respect to the rearing condition of the males. Our results suggest that under some conditions females choose compatible mates, however these choices may be altered by interactions with the male.

Keywords: Chicken; Sexual selection; Female choice; Ontogeny

1. Introduction

In many species of birds, females mate non-randomly, often showing preferences for traits associated with male condition or quality (e.g. Weatherhead and Robertson, 1979; Hill, 1991; Johnson et al., 1993). These traits may have an indirect impact on female

^{*} Corresponding author at: Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1, Canada. Tel.: (902) 494-2158; fax: (902) 494-3736.

¹ Present address: Department of Ecosystem Management, University of New England, Armidale, N.S.W. 2351, Australia

² Present address: Department of Zoology, University of British Columbia, Vancouver, B.C. V6R 3B1, Canada

fitness if males provide 'good genes' to their offspring. Alternatively, males could also have a direct effect if they provide resources such as parental care or protection against predators. In some species, the interaction of male and female traits may also influence reproductive success (e.g. Coulson, 1972; Rowley, 1983; Leonard et al., 1993b). Whatever the case, females are generally expected to choose mates with qualities that enhance their fitness.

Female red junglefowl (*Gallus gallus murghi*) prefer males with long, red combs and red irises (Zuk et al., 1990a, Zuk et al., 1992; Ligon and Zwartjes, 1995a). These traits depend on the male's health and thus should provide females with reliable information about the condition of the male (Zuk et al., 1990b). Although relatively little is known about mate choice in domestic chickens, early studies suggest that female chickens may also mate non-randomly (e.g. Guhl, 1951; Graves et al., 1985).

Male White Leghorn chickens exposed to females during rearing are more sexually active than males reared in all male groups (Siegel and Siegel, 1964; Leonard et al., 1993a). Furthermore, pairs of males and females with similar early experience (i.e. both reared either with or without the opposite sex) have higher mating success and initially produce more eggs than pairs with different rearing conditions (Leonard et al., 1993b). This suggests that the compatibility of the pair in terms of their previous experience with the opposite sex is an important factor in mating success (see Leonard et al., 1993b for discussion). Thus we predicted that when given a choice, females should prefer to mate with males with similar early experience (i.e. compatible males).

The purpose of our study was to use mate choice tests to determine whether female White Leghorn chickens reared under sex-separate and sex-mixed conditions displayed a preference for males reared under the same conditions as themselves.

2. Methods

2.1. Rearing conditions

At hatch, 420 female and 100 male White Leghorn chickens were randomly assigned to one of three all-female (140 females per pen; single-sex condition) or two all-male pens (50 males per pen; single-sex condition). In addition, 560 females and 48 males were assigned to one of four male–female pens (12 males and 140 females per pen; mixed-sex conditions). Each pen was 3.65×5.10 m with a littered floor area at the front containing two suspended feeders and a roosting area at the rear with two bell drinkers.

Because we were interested in the effect of early experience on mate choice, we separated males and females in mixed-sex pens at 18 weeks to prevent them from gaining adult sexual experience. These birds were placed in either one of four all-male (12 males per pen) or eight all-female (70 females per pen) pens. Individuals of both sexes were placed with birds from their original pens. This separation occurred as males were beginning to show courting behaviour, but before we observed copulations and before females began to lay. Males and females in the single-sex conditions were also moved at 18 weeks to one of three all-male pens (15 males per pen) or one of six all female pens (70 females per pen).

The photoperiod was gradually decreased from 23L:1D at Day 1 to 9L:15D by Week 7, after which it was constant until the end of the rearing period at 18 weeks. Then the photoperiod was changed to 11L:13D and increased weekly until it reached 14L:10D at 30 weeks. Food and water were provided ad libitum throughout the study.

2.2. *Morphometrics*

Morphological characteristics, such as comb colour and size and iris colour, are important traits in mate choice in female red junglefowl (e.g. Zuk et al., 1990b; Ligon and Zwartjes, 1995a). Therefore, we measured comb length and width (cm), wattle length and width (cm), body weight (kg) and tarsus length (cm) for each male when it was moved, at 18 weeks of age. We also took colour photographs of the head of each male under identical lighting conditions and then used a Transmission densitometer (Macbeth TR527) to determine the density of red, blue and green pigments in the comb and right iris.

2.3. *Experimental conditions*

Two pens were modified by dividing the rear half of each pen into two 1.82×2.29 m compartments separated by a 2.29-m high metal partition. The remaining 3.65×2.81 m area in the front half of each pen was covered in litter and separated from each compartment by a chicken wire gate which could be raised and lowered from outside the pen. Males were tethered in the centre of each compartment by a 2.27 m-long cotton lead that was attached to the ceiling. The tether was hooked to an elastic loop that was attached to a small cotton patch glued to the feathers of the lower back. Observations from pilot trials indicated that once accustomed to the tether, males moved normally and displayed typical courtship behaviour.

Males could move freely throughout the entire compartment with the exception of a 50-cm wide strip at the front edge. In this section, females could enter the compartment, but remain outside the reach of the male. Males in separate compartments could hear, but not see each other.

2.4. *Choice trials*

Mate choice trials were conducted between 08:00 and 14:00 daily for 8 days beginning when males and females were 23 weeks old. At this age, males were showing the full repertoire of courting and mating behaviour and females were laying eggs.

Twenty-four hours before a trial, up to five pairs of males (five males from each rearing condition) were randomly chosen from their home pens and tethered in each of five holding pens (i.e. one pair of males per holding pen). Each pen was identical to the experimental pens except that males had access to food and water and compartments did not have gates. This procedure allowed the males to become accustomed to the experimental set-up and to moving on the tether.

The following day a randomly chosen male from each rearing condition was tethered in an experimental compartment with the gates closed. Males from each rearing

condition were alternated between the left and right compartment between trials. This resulted in single-sex and mixed-sex males appearing on the right and left side an equal number of times. In addition, the mixed-sex males used in the choice trials were not familiar to the mixed-sex females (i.e. they had not been reared in the same pen).

Females from each rearing condition were selected at random from their home pens immediately before each trial. Trials using single-sex females were alternated with trials using mixed-sex females. In total, we conducted 20 trials with females from each rearing condition for a total of 40 trials (one trial was excluded because a male's tether broke). Each male and female was used in one trial only. After the trial was complete, the patch was removed from the male's feathers and males and females were returned to their home pens.

During a trial, an observer sat outside the experimental pen. Trials were also videotaped for later analyses. A trial consisted of a 5-min period before videotaping during which the males became accustomed to the presence of the observer, followed by: (i) 5 min during which the behaviour of each male was recorded in the absence of the female (Stage 1); (ii) 5 min during which the female was placed in a wire enclosure in the centre of the littered area, equidistant from each male compartment (Stage 2); (iii) 10 min in which this enclosure was lifted from outside the pen and the female had access to the littered area (Stage 3) and (iv) 20 min during which the gates to the male compartments were opened and the female had access to each male (Stage 4).

We recorded the behaviour of the males during the portion of the trial in which the female was present and equidistant from each male (i.e. Stage 2) and when the female was in their compartment (Stage 4). During Stage 2, we also recorded the amount of time each male spent in either the front or back half of their compartment.

When females were released from the enclosure we recorded the amount of time they spent in the: (i) back (section closest to the male) left quarter of the littered area; (ii) back right quarter; (iii) front left quarter and (iv) front right quarter. Once the gates were opened (i.e. Stage 4), we also recorded the amount of time they spent in each male compartment.

2.5. Preference measures

We examined female preferences under two conditions; when females were free, but could not interact directly with the males (Stage 3) and when females could approach and physically interact with the males (Stage 4). We included analyses of both stages because it allowed us to examine the influence of interactions with the male on female preference. Female preference was based on: (i) the amount of time the female spent on either the right or left side of the pen (time in the front + time in the back on each side) during Stage 3 and (ii) the amount of time the female spent in the back plus the time spent in a male's compartment during Stage 4. We used time spent as a measure of preference because copulations are relatively infrequent (mean: 1.32 copulations h^{-1} ; total in this study: 4; Leonard, unpublished) and because it was the only measure we could use in Stage 3. The results of a choice experiment in which females had twice as much time to interact with males as in the present study showed that females spent significantly more time in the compartment of the male they eventually copulated with

($F = 23.22$, d.f. = 2,65, $P = 0.001$; Leonard, in preparation). Therefore, time spent with a male is a reliable measure of preference.

A female was considered to have preferred a male if she spent more time with that male than she did with the other male. If the difference in the amount of time spent with both males was less than 20% of the total observation time for that stage we assumed that the female did not have a preference. Therefore, these trials were not included in the analyses examining female preferences. We considered this to be a conservative measure of preference and inclusion of all trials did not change the results. During Stage 4, we also used the identity of the male whose compartment was entered first when the gates were opened as another measure of preference.

We controlled for the amount of time females spent in the compartment of different males when comparing the behaviour of the males during Stage 4. Means are reported \pm one standard error. Statistical significance is set at $P \leq 0.05$ and P values greater than 0.10 are reported as NS.

3. Results

3.1. Male morphology and behaviour

With the exception of weight, body measurements of single-sex and mixed-sex males were not significantly different (Table 1). Single-sex males did, however, weigh significantly more than mixed-sex males (Table 1). Comb and iris colour did not differ significantly for males from different rearing conditions (unpaired t -tests, all NS).

Mixed-sex males spent significantly more time at the front of their compartment and wing-flapped significantly more often than single-sex males during Stage 2 (i.e. female present, but enclosed; Table 2). Otherwise, the behaviour of the males did not differ during this stage (Table 2). During Stage 4, single-sex males crowded at a significantly higher rate than mixed-sex males when the female was in their compartment (paired-test, $t = 2.0$, d.f. = 22, $P = 0.05$), but otherwise males did not differ in their behaviour (paired t -tests, NS).

Table 1

Mean (\pm SE) comb length (cm), comb width (cm), wattle length (cm), wattle width (cm), tarsus length (cm) and body weight (kg) for mixed-sex and single-sex males at 18 weeks of age

Measurements	Mixed-sex	Single-sex	t	P
Comb length	10.9 \pm 0.19	11.0 \pm 0.16	0.22	NS
Comb width	6.14 \pm 0.11	6.02 \pm 0.09	-0.81	NS
Wattle length	4.69 \pm 0.12	4.62 \pm 0.11	-0.46	NS
Wattle width	3.97 \pm 0.09	4.11 \pm 0.09	1.08	NS
Tarsus length	11.2 \pm 0.06	11.2 \pm 0.06	0.30	NS
Body weight	1.75 \pm 0.02	1.81 \pm 0.01	2.05	0.04

Means were compared using an unpaired t -test (78 df).

Table 2

Mean (\pm SE) number of crows, wing-flaps and time spent at the front of the compartment (min) for mixed-sex and single-sex males when the female was enclosed in a wire cage which was equally visible to both males

Behaviour	Mixed-sex	Single-sex	<i>t</i>	<i>P</i>
Crows	3.03 \pm 0.54	3.30 \pm 0.54	0.35	NS
Wing-flap	3.45 \pm 0.49	2.45 \pm 0.38	-2.26	0.03
Time front	4.41 \pm 0.16	3.71 \pm 0.27	-2.09	0.04

Means were compared using a paired *t*-test (39 df).

3.2. Female preference

During Stage 3 (i.e. female free, but gates closed), females had preferences for particular males in 25 of 39 trials. In 11 of the 14 trials in which single-sex females showed a preference, they selected a single-sex male and in eight of 11 trials in which mixed-sex females had a preference they chose a mixed-sex male. Single-sex and mixed-sex females showed no significant difference in how often they chose a male with the same rearing condition as their own ($X^2 = 0.10$, d.f. = 1, NS). Overall, females chose males whose rearing condition matched their own significantly more often than predicted by chance (19/25, Binomial test, $P < 0.05$).

During Stage 4 (i.e. gates open), females had preferences in 19 of 39 trials. Single-sex females preferred single-sex males in seven of 11 trials while mixed-sex females chose mixed-sex males in four of eight trials. Again, females from each rearing condition showed no significant difference in their patterns of mate choice ($X^2 = 0.4$, d.f. = 1, NS). Overall, females showed no significant preference for males from the same rearing conditions as their own (11/19, Binomial test, NS). To examine whether this result was a function of the difference in the length of Stages 3 and 4 (10 min versus 20 min), we restricted our analyses to the first 10 min of Stage 4. During these first 10 min females showed preferences in 32 of 39 trials with females from each rearing condition showing similar patterns of choice ($X^2 = 1.0$, d.f. = 1, NS). However, as in the analysis of the entire 20 min trial, they chose males from rearing conditions matching their own no more often than expected by chance (14/32, Binomial test, NS).

Single-sex females entered the compartment of a single-sex male first in 10 of 19 trials (Binomial test, NS) and mixed-sex females entered the compartment of a mixed-sex male first in 11 of 20 trials (Binomial test, NS).

Females may have based their choices on features of males that were not associated with rearing condition. However, males that were chosen did not differ from males that were not in either their morphology or behaviour (paired *t*-tests, all NS).

4. Discussion

Breeding success may be based not only on the individual attributes of each partner, but also on the compatibility of the pair (Coulson, 1972; Rowley, 1983). For instance, in Kittiwake gulls (*Rissa tridactyla*) both males and females incubate and the compatibility

of the pair in terms of the incubation schedule can have a major impact on hatching success (Coulson, 1972). Our recent study suggested that compatibility also had an important influence on mating success in layer parent stocks (Leonard et al., 1993a). Although we did not identify the factors that affected compatibility, we did show that early experience with the opposite sex produces behavioural and morphological differences, which presumably affect interactions between males and females and thus, mating success (Leonard et al., 1993a, Leonard et al., 1993b).

Based on the results of these studies, we predicted that female White Leghorns should prefer to mate with compatible males (i.e. males with similar early experience). If this is the case, then females need reliable cues with which to assess the potential compatibility of the male. In our earlier study, mixed-sex males showed more sexual behaviour and spent more time near a model of a female than single-sex males, while single-sex males were more aggressive and crowded more in the presence of the model (Leonard et al., 1993b). Similarly, in the present study mixed-sex males spent more time near the front of their compartments (i.e. closer to the female) and showed more wing-flapping (i.e. courting behaviour, Wood-gush, 1956) while single-sex males crowded more often when a female was in their compartment. Thus behavioural differences between the males were consistent across the two studies, suggesting that they may be reliable indicators of early experience. Morphological traits associated with the two rearing conditions, however, differed between the studies. Single-sex males in the earlier study had longer combs and wider wattles than mixed-sex males (Leonard et al., 1993b), but in this study, these features did not differ and instead single-sex males were heavier than mixed-sex males. These results suggest that behavioural, but not morphological features could be used by females to assess the previous experience, and thus compatibility, of the males.

As predicted, the females in this study initially showed a preference for males with similar early experience. However, this preference disappeared when the females were free to interact with the males. It is not clear why this was the case. One possibility is that the behaviour of the males altered female preferences. For example, some males lunged at females as they entered the compartment often driving the female away. Single-sex males did not lunge more often than mixed-sex males. However, the frequency of lunging was twice as high among males that were rejected than it was among males that were chosen, although the difference was not significant (rejected: 0.36 ± 0.22 ; chosen: 0.17 ± 0.13). This suggests that females may have avoided males that were particularly aggressive, thus affecting their original preference. Another, more interesting possibility is that the behaviour of the males changed once they interacted with the female, making it difficult for the female to assess their rearing status. If this were the case, females would be expected to choose males at random (e.g. Ligon and Zwartjes, 1995b). This suggests that in a more realistic situation where females interact directly with males the necessary cues may not be available.

The difference in response by females in the two situations has implications for the design of mate choice experiments. Typically in choice tests, females are free to interact directly with males (e.g. Zuk et al., 1990a; Ligon and Zwartjes, 1995a, Ligon and Zwartjes, 1995b). However, in some studies females have no direct contact with the males (e.g. Johnson et al., 1993). Our results suggest that both components should

probably be included in future studies if we are to understand the factors influencing mate choice.

Interestingly, many females did not show a strong preference for either male, but rather spent similar amounts of time with each. In a series of mate choice trials, Ligon and Zwartjes (1995b) found that female red junglefowl often mated with both males even when there were large differences in a preferred trait such as comb size. One explanation for this apparent lack of preference was that females, in addition to mating with high quality males, were also attempting to increase the genetic diversity of their brood (Ligon and Zwartjes, 1995b). Whatever the reason, the results of both studies suggest that the reproductive strategy of female fowl may include mating with more than one male.

In conclusion, female White Leghorn chickens showed preferences for males with similar early experience. Presumably this is because females benefit from mating with compatible males. However, this preference was only obvious before females had direct contact with males suggesting that interactions with the males affected their choice. Future studies should probably be designed to allow females to make choices under both conditions, so that the influence of the male on female choice can be assessed.

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References

- Coulson, J.C., 1972. The significance of the pair-bond in the Kittiwake. In: *Proceedings of the XVth International Ornithological Congress*, Brill, Leiden, pp. 424–33.
- Graves, H.B., Hable, C.P. and Jenkins, T.H., 1985. Sexual selection in *Gallus*: effects of morphology and dominance on female spatial behaviour. *Behav. Proc.*, 11: 189–197.
- Guhl, A.M., 1951. Measurable differences in mating behaviour of cocks. *Poult. Sci.*, 30: 687–693.
- Hill, G.E., 1991. Plumage color is a sexually selected indicator of male quality. *Nature*, 350: 337–339.
- Johnson, K., Dalton, R. and Burley, N., 1993. Preferences of female American goldfinches (*Carduelis tristis*) for natural and artificial male traits. *Behav. Ecol.*, 4: 138–143.
- Leonard, M.L., Zanette, L. and Fairfull, R.W., 1993a. Early exposure to females affects interactions between male White Leghorn chickens. *Appl. Anim. Behav. Sci.*, 36: 29–38.
- Leonard, M.L., Zanette, L., Thompson, B.K. and Fairfull, R.W., 1993b. Early exposure to the opposite sex affects mating behaviour in White Leghorn chickens. *Appl. Anim. Behav. Sci.*, 37: 57–67.
- Ligon, J.D. and Zwartjes, P.W., 1995a. Ornate plumage of male red junglefowl does not influence mate choice by females. *Anim. Behav.*, 49: 117–125.
- Ligon, J.D. and Zwartjes, P.W., 1995b. Female red junglefowl choose to mate with multiple males. *Anim. Behav.*, 49: 127–135.
- Rowley, I., 1983. Re-mating in birds. In: P. Bateson (Editor), *Mate Choice*. Cambridge University Press, Cambridge, pp. 331–360.

- Siegel, P.B. and Siegel, H.S., 1964. Rearing methods and subsequent sexual behaviour of male chickens. *Anim. Behav.*, 12: 270–271.
- Weatherhead, P.J. and Robertson, R.J., 1979. Offspring quality and the polygyny threshold: “The sexy son hypothesis”. *Am. Nat.*, 113: 201–208.
- Wood-gush, D.G.M., 1956. The agonistic and courtship behavior of the Brown Leghorn cock. *Br. J. Anim. Behav.*, 4: 133–142.
- Zuk, M., Ligon, J.D. and Thornhill, R., 1992. Effects of experimental manipulation of male secondary sex characters on female male preference in red jungle fowl. *Anim. Behav.*, 44: 999–1006.
- Zuk, M., Thornhill, R., Ligon, J.D., Johnson, K., Austad, S., Ligon, S.H., Thornhill, N.W. and Costin, C., 1990a. The role of male ornaments and courtship behaviour in female mate choice in red jungle fowl. *Am. Nat.*, 136: 459–473.
- Zuk, M., Thornhill, R. and Ligon, J.D., 1990b. Parasites and mate choice in red jungle fowl. *Am. Zool.*, 30: 235–244.