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# Do domestic hens discriminate between familiar and unfamiliar conspecifics in the absence of visual cues?

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## Abstract

Domestic fowl (*Gallus gallus*) are able to discriminate between familiar and unfamiliar conspecifics, at least if they may rely on visual, auditory and olfactory cues from live stimulus birds. The Red Junglefowl, the ancestor of the domestic fowl, inhabits woody and bushy areas and the edge of jungles where sight is restricted by dense vegetation. Consequently non-visual communication is more suited to such an environment than is visual communication. Individual non-visual recognition would enable the birds to maintain their group and social structure despite the complex nature of the environment. In the present study, we test if domestic chickens do discriminate between familiar and unfamiliar conspecifics in the absence of visual cues.

We carried out two preference experiments. In each test, two live stimulus hens, one familiar and one unfamiliar, were presented to a test hen. In the first experiment, the hens were separated only by wire-mesh, allowing the test hen to receive visual as well as auditory and olfactory stimuli from the stimulus hens. In the second experiment, visual contact between the hens was prevented by replacing the wire-mesh with an opaque black cloth. As a measure of preference the aggregation time with the familiar and the unfamiliar bird was recorded.

In the first experiment, hens showed significant discrimination between familiar and unfamiliar conspecifics. However, hens in two experimental series showed different directions of preference. In the second experiment, where test hens had to rely on non-visual cues, no significant discrimination between familiar and unfamiliar conspecifics was observed.

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## 1. Introduction

The Red Junglefowl is a highly social animal, which forms a cohesive social structure (Wood-Gush, 1971). It establishes largely stable dominance hierarchies that are thought to reduce aggression. In small groups, hierarchies are often linear, whereas in larger groups triangle and polygon relationships may be observed (Wennrich, 1978). For establishing linear hierarchies, it is sufficient that an individual is able to distinguish its conspecifics as being more dominant or less dominant than itself, but for more sophisticated social structures individual recognition between group members is a prerequisite. Group living in Red Junglefowl is characterised by dominant males setting up territories with harems in the habitat most favoured by the females (Johnson, 1963, but see also Collias and Collias, 1996). A group contains up to 30 adult birds that rarely move more than 50 m from their home roost. Groups are fairly closed. Some interchanges between neighbouring flocks do occur, but the numbers involved are low when compared to the increase in progeny and the losses through mortality (Collias and Collias, 1996).

From the description of the social structure of the Red Junglefowl, one may conclude that individuals have the ability to recognise conspecifics individually and to distinguish between flock members and strange birds. Such recognition may be based on visual, auditory or olfactory cues. Several studies have shown that chickens are able to distinguish between familiar and unfamiliar conspecifics using at least visual, auditory and olfactory cues from live stimulus birds. In an arena containing four stimulus hens, test birds spent significantly more time in front of the familiar than in front of the unfamiliar conspecifics (Bradshaw, 1992), and in a runway test, birds took longer to reach the target cage in the presence of an unfamiliar bird than in presence of a familiar one (Grigor et al., 1995). Furthermore, social affiliation was more pronounced when the hens were confronted with a cage mate rather than with a strange chick (Marin et al., 2001). However, discrimination was not successful when hens were further than 30 cm apart, the range in which chickens are able to focus (Stamp Dawkins, 1995). Additionally, hens failed to distinguish between familiar and unfamiliar birds under red or blue illumination (D'Eath and Stone, 1999). Both studies underline the importance of visual cues. Olfactory cues may play a less important role. Generally, birds are thought to have an under-developed sense of smell, but it should not be overlooked. Experiments have shown that chicks are able to distinguish between familiar and unfamiliar soiled substrate (Jones, 1982), and that they have a preference for aromatic oils they are acquainted with (Jones, 1984). To our knowledge, the auditory aspects of individual and group recognition in chickens have not received much attention, even though the physiological conditions for a possible acoustic recognition exist. Temple et al. (1984) states that the range of sensitive hearing in chickens corresponds with the range of dominant frequencies reported in most studies of hen calls. In work on the relationship between chicks and the parent hen, Kent (1987) showed that chicks are able to recognise their parent hen by means of the hen's cluck vocalisations.

The vocal repertoire of the domestic fowl is essentially the same as in the Red Junglefowl. Twenty-four signals are described, but because of integration of some signals and the occurrence of different situations, the precise vocal repertoire is unknown (Collias, 1987). Calls are important in synchronising activities, but vocalisation does not seem to be important in maintaining the peck-order (Wood-Gush, 1965; Wood-Gush, 1971). Experimental evidence

for the function of a particular call, the gakel call, was found by [Zimmerman and Koene \(1998\)](#) in relation to frustration. Another study showed that vocalisation is strongly dependent on social contact between chicks. Total vocalisation and the pattern of calls changed significantly during stepwise isolation ([Marx et al., 2001](#)).

Last but not the least, there is also an evolutionary argument as to why we would expect non-visual cues to be important for individual recognition in chickens. The Red Junglefowl, as the ancestor of the domestic fowl, inhabits woody and bushy areas and the edge of jungles where sight is restricted by dense vegetation. Auditory communication is especially suited to such an environment and we may expect it to be as important as visual communication. Individual auditory recognition would enable the chickens to maintain their group and social structure despite the complex nature of the environment. In the present study, we test if domestic chickens do discriminate between familiar and unfamiliar conspecifics in the absence of visual cues.

We carried out two preference experiments. In each test, two live stimulus hens, one familiar and one unfamiliar, were presented at the opposite ends of a choice area containing the test hen. The first experiment intended to assure that the experimental set-up is appropriate to investigate preference behaviour. The hens were separated only by wire-mesh, allowing the test hen to receive auditory, olfactory as well as visual stimuli from the stimulus hens. In the second experiment, the wire-mesh was replaced with an opaque black cloth removing visual cues, but allowing auditory and olfactory ones to pass. Preference tests may be influenced, among other things, by the dominance status of the test bird and by its fearfulness. We, therefore, assessed the dominance status of the birds by recording body weight and the surface area of the comb. Fearfulness of the test birds was assessed by measuring the duration of the tonic immobility reaction.

## **2. Method**

### *2.1. Animals and husbandry*

In the first experiment, 36 ISA Brown laying hens reared by a commercial breeder served as experimental animals. They were taken from a flock of 2000 birds 11 weeks after hatching. After the light was switched off in the evening, 36 hens were chosen to form four experimental groups (four groups of nine). Groups were composed of roosting-neighbours that were taken, for each group, from the most distant locations within the system. Each group was put in a box (0.6 m × 0.4 m × 0.4 m) and brought to the institute. At the institute, all birds were individually marked with coloured leg bands and housed in identical pens at a density of 1.2 hens/m<sup>2</sup>. Pens were equipped with a perch (2.5 m long, 0.8 m high) and a dust bath (0.9 m × 0.5 m). The birds had free access to feed and water. The floor was covered with a mixture of wood shavings, straw and chaff. The light–dark schedule was set to 9:15 h as recommended by commercial rearing programs. Light intensity at the height of the birds was 50 lx and the ambient temperature 18 °C. In order that they became familiar with each other, the birds spent 5 weeks together in their groups before the experiment started at Week 16.

In the second experiment, 80 hens served as experimental animals (eight groups of 10 birds). They were collected at Week 9 as described for Experiment 1 and kept at a density of

2.0 hens/m<sup>2</sup>. Again they were allowed 5 weeks to become familiar with each other before the experiment started at Week 14. After the termination of the experiments the birds were sold to commercial poultry farmers.

## 2.2. Experimental apparatus

The birds were monitored in a rectangular box made of plywood (2.5 m × 1 m × 0.7 m) consisting of a central ‘choice’ area (1.5 m long) for the test bird and two outer chambers (0.5 m long) for the familiar and the unfamiliar stimulus birds (Fig. 1). The chambers were separated by a dividing wall of wire-mesh (1.7 cm × 1.7 cm mesh size) in Experiment 1 or of opaque black cloth in Experiment 2. With a frame of wire-mesh (5 cm × 5 cm mesh size) the apparatus could be closed and opened. The floor of the box was covered with wood shavings and was cleaned and the shavings redistributed after each trial. As of the longer duration of the test in Experiment 2, bowls with water were placed in front of the dividing walls. The water was changed after each trial.

As a ‘starting box’ we used a cylindrical construction placed in the centre of the ‘choice’ area. It was made of black cloth (45 cm high) stretched onto two metal rings (45 cm diameter). On the top of this cylinder a cover of wire-mesh (5 cm × 5 cm mesh size) prevented the hens from escaping. By pulling up the lower ring, the cloth was lifted up and the hen could move freely around the choice area.

The box was illuminated by two fluorescent lights that could be dimmed. The lights were operated from the adjoining room.

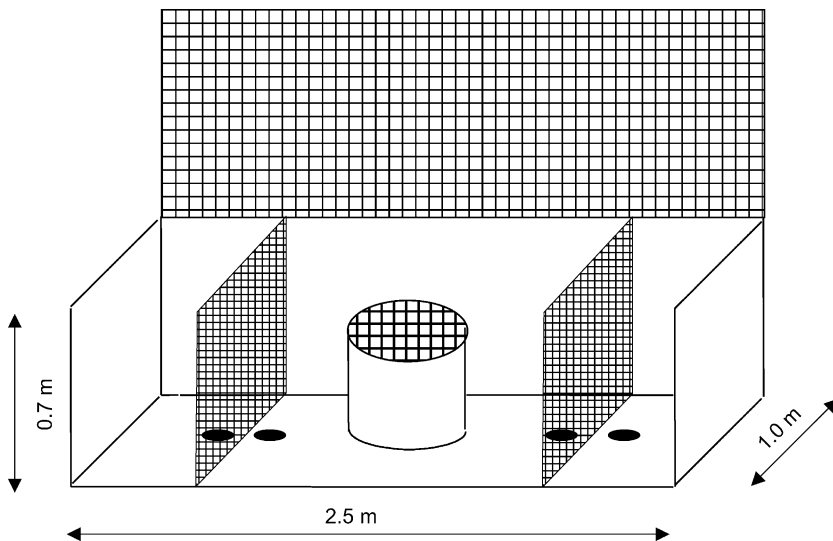


Fig. 1. Experimental box consisting of a central choice area for the test bird and two outer chambers for the familiar and unfamiliar stimulus birds. The chambers were separated by dividing walls of wire mesh (Experiment 1) or black cloth (Experiment 2). The cylindrical start box in the middle of the central area could be pulled up. Water bowls (shown as oval spots) were placed on both sides of the dividing walls (Experiment 2).

The test box was enclosed by opaque curtains to assure that no visual impact from outside could reach the birds in the apparatus. In addition, the experimental room was divided from the pens by two rooms so that there was no auditory contact between the experimental room and the pens.

All trials were taped on video using overhead cameras. Vocals were recorded through a central microphone fixed above the apparatus. In this way, the test box could be optically and acoustically controlled from the adjoining room.

### 2.3. Procedures

#### 2.3.1. Experiment 1

**2.3.1.1. Habituation.** On two occasions, the birds were placed in the test box and handled. A first habituation was carried out 3 weeks before the experiment began. The dividing walls of wire-mesh were already present but shifted to the sidewalls, so that the central chamber was fully expanded. The cylindrical start box and the fluorescent lights were not yet installed. Each of the 36 hens was set in the middle of the experimental apparatus parallel to the sidewalls (the head was alternately oriented to the back or the front wall) and was then allowed to move freely. The experimenter closed the curtains, left the room and observed the bird on a screen in the adjoining room for 20 min. To check for possible bias in the experimental set-up, the time birds spent on the left and right half of the choice area was recorded, but no detectable bias was found (Student's *t*-test:  $t = 0.538$ ,  $N = 30$ ,  $P = 0.595$ ). A second habituation took place in the afternoon 1 day before the trial and lasted 10 min. It was carried out with the dividing walls of wire-mesh in place as for Experiment 1 (Fig. 1) and with the use of the starting box as well as the adjustable lights (see below), but still without any stimulus hens.

**2.3.1.2. Preference test.** Two stimulus birds were caught and put separately into cardboard boxes in order to transfer them to the experimental room. One stimulus hen was a group member (familiar) and the other stimulus hen was from another group (unfamiliar). The familiar and unfamiliar hens were set first into the left and right outer chamber of the test box. Order and side of the outer chambers were alternated between trials. Afterwards the test hen was caught and set into the starting box. The coloured leg bands were removed previous to the trials to avoid possible colour preferences (Hurnik et al., 1973; Hurnik et al., 1977).

The stimulus birds as well as the test hen were set into the box in darkness. The light was then slowly turned on, the starting box was pulled up and the test hen could move freely around the central 'choice' area for 20 min. At the end of the trial, the light was dimmed to facilitate the catching of the birds. Time taken for the different steps of the procedure (catching, transfer to the test box, turning on the lights, lifting up the start box, lowering the lights at the end) was kept constant over all trials. All trials took place between 09:00 and 13:00 h.

Preference tests were recorded on video from which we later measured the amount of time that the test hen spent within 25 cm of the front of the walls dividing it from the stimulus birds. This amount of time is called 'aggregation time' with the familiar or unfamiliar conspecific. A black point (2 cm in diameter) sprayed on the back of the test hen

served as a point of measure when deciding whether the test hen was within the 25 cm or not.

To increase sample size, some birds were used twice. This was done in a controlled way to allow later analysis of possible effects from using the birds more than once.

Trials 1–20 (Series 1) involved test hens that were totally naive to the test situation and the stimulus hens of a specific trial had the same ‘history’. That means that both stimulus hens in a trial were either naive (eight trials), had already been used once as the stimulus hen (four trials) or as the test hen (eight trials).

Trials 21–36 (Series 2) involved test hens that had already been used once as stimulus hens. Again, stimulus hens in a specific trial had the same ‘history’, i.e. both stimulus hens had already been used once as test hens (two trials); both had been used once as stimulus hens (five trials); or both had been used previously both as stimulus hens and test hens (nine trials). Due to operational reasons (the pens where we kept the birds were no longer available), we had to increase the number of trials per day and consequently half of the trials in Series 2 were run between 13:00 and 16:00 h.

*2.3.1.3. Indirect measurements of dominance.* In Experiment 1, we measured body weight and the surface area of the comb at Week 17, to assess the influence of dominance status on the results of the preference test. Both measurements are known to correlate with the dominance status (Collias, 1943). Combs were photographed with a digital camera in a defined way (Nikon E2 with two flashes Nikon SB26) in front of black velvet and comb area was then determined with the help of density slicing using ‘Scion Image for Windows’.

### *2.3.2. Experiment 2*

*2.3.2.1. Habituation.* During the habituation procedure in Experiment 1, the hens showed few signs of fear when put into the test box. They quickly started to explore the entire box. It was therefore decided that under these circumstances habituation was not necessary and no habituation trials were carried out in Experiment 2.

*2.3.2.2. Preference test.* The procedure was essentially the same as in Experiment 1, except that the dividing walls were made of black cloth instead of wire-mesh, so that the test hen and the stimulus hens could not see each other. Differences were found between Series 1 and 2 in Experiment 1, so we increased the number of groups to eight, which allowed us to exclusively use naive test hens. In order to take into account a possible reduction in the effects found in Experiment 1 due to the absence of optical contact between the hens, we increased the sample size to 48 trials. In an additional pilot study with hens from Experiment 1, we optimised the duration of the trials, having found that an observation time of 90 min gave clearer preferences than 20 min. The trials were run at 09:30, 12:00 and 14:30 h each day for 16 days. In Experiment 1, we used a distance of 25 cm as evidence of preference because beyond this distance visual recognition may be difficult (Stamp Dawkins, 1995). As there were no visual cues available to the hens in Experiment 2, we used aggregation time in the left or right half of the choice area as evidence of preference.

All the test hens were naive and the stimulus birds were either naive (Series n); had once been used as stimulus hens (Series st); or had once been used as test hens (Series t). Care was taken that the two stimulus birds in a specific trial had the same history. All series

consisted of 16 trials that were randomly distributed over the three times when trials took place each day.

**2.3.2.3. Tonic immobility (TI) test.** Fearful birds might show a tendency to aggregate with familiar birds when confronted with an unknown situation. We, therefore, recorded individual levels of fearfulness by measuring the duration of the tonic immobility reaction (Jones, 1987). We used a U-shaped cradle that was located in a box made of plywood (1 m × 1 m × 1 m) with an open front. The front was covered by two black curtains, overlapping in the middle. The floor was covered with wood shavings, chaff and straw as in the home pens and the box was illuminated using two fluorescent lights. With the help of an overhead camera, the test bird could be observed on a screen next to the box (to control the induction) and on an additional screen in the adjoining room. After each trial the stimulus birds were brought back to their home pens and the TI reaction of the test hen was determined. The TI test was carried out 4 min after the end of the trial and in the same room. At the time of the TI test, the experimenter was not aware of the bird's choice in the preference test, as data were collected from videotapes after the TI test.

TI was induced by laying the bird down on its back in the cradle. A hand was placed on the chest to restrain the bird and it was observed on the screen, whilst the curtains remained closed, so that the bird could not see the experimenter. After 45 s the hand was withdrawn and the experimenter left the room. The bird was monitored on the screen in the adjoining room until the end of the tonic immobility. The time was measured from withdrawal of the hand until the bird straightened up. The procedure was taped on video to allow further analysis later on, if necessary.

Birds showing unclear tonic immobility (<10 s) were excluded from the analysis (two hens). If a bird did not straighten up within 15 min, it was removed and given the maximal score of 900 s (eight hens).

#### 2.4. Analysis and statistics

Data were checked for normality (Shapiro-Wilk) and transformed (square root), if necessary. Residuals were tested for normal distribution in ANOVA models and, if necessary, data were transformed (square root) to achieve normally distributed residuals. All *P*-values are two-tailed. Trials in which test hens did not go within 25 cm of the dividing walls during the total observation time (aggregation time = 0 s) were excluded from the analysis (Experiment 1).

Whether factors other than the familiarity had an effect on aggregation time with the stimulus birds was assessed before data were pooled within an experiment. In Experiment 1, the identity of the test hen's group and the series (see above Section 2.3.1.2) were considered. The former did not influence aggregation time, neither with the familiar nor the unfamiliar stimulus bird (ANOVA:  $F_{3,36} = 1.399$ ,  $P = 0.262$  and  $F_{3,35} = 0.409$ ,  $P = 0.747$ , respectively), but the factor 'series' significantly affected aggregation time, both with the familiar and the unfamiliar stimulus bird (ANOVA:  $F_{1,36} = 9.489$ ,  $P = 0.004$  and  $F_{1,35} = 4.351$ ,  $P = 0.045$ , respectively). Aggregation times were, therefore, analysed separately for each series. In the second experiment, in addition to the identity of the test hen's group and the factor 'series', the time of day when the trials were conducted was also considered,

since in Experiment 1 an influence of the time of day on aggregation times could not be excluded. None of the three factors had a significant effect on aggregation times with familiar or unfamiliar stimulus birds (ANOVA: group:  $F_{7,45} = 0.806$ ,  $P = 0.589$  and  $F_{7,44} = 0.713$ ,  $P = 0.662$ , respectively; series:  $F_{2,45} = 0.945$ ,  $P = 0.399$  and  $F_{2,44} = 0.510$ ,  $P = 0.605$ , respectively; time of day:  $F_{1,45} = 2.015$ ,  $P = 0.165$  and  $F_{1,44} = 1.603$ ,  $P = 0.214$ , respectively). Data from all trials in Experiment 2 were therefore pooled.

To test whether hens discriminate between familiar and unfamiliar hens, the aggregation times with the familiar and unfamiliar stimulus hen were compared using a paired *t*-test. Additionally, by using Chi-Square tests we checked if hens spent more or less time than expected in the 25 cm in front of the dividing wall when compared to the rest of the choice area.

Vocal behaviour during the trials in Experiment 2 was analysed in a semi-quantitative way. Total time of observation was divided into 18 intervals of 5 min, and for each interval we recorded whether there were calls given or not. The development of calling activity over time was investigated using a page test for ordered alternatives (Siegel and Castellan, 1988).

Power and effect size of the experiments were calculated according to Cohen (1988) and significance of correlation coefficients were checked using the *F*-statistic (Zar, 1999). To perform the analyses, we used the statistical program JMPIn 3.2.1 (SAS Campus Drive, Cary, NC).

### 3. Results

#### 3.1. Experiment 1

The hens of both series spent significantly more time than expected within 25 cm of the dividing walls (Series 1:  $\chi = 147.02$ ,  $P < 0.001$ ,  $N = 20$ ; Series 2:  $\chi = 104.33$ ,  $P < 0.001$ ,  $N = 20$ ).

The hens of Series 1 spent on average  $207.1 \pm 103.6$  s ( $N = 20$ ) in front of the familiar and  $359.7 \pm 185.0$  s ( $N = 20$ ) in front of the unfamiliar hen. This difference was statistically significant (paired *t*-test:  $t = -3.181$ ,  $N = 20$ ,  $P = 0.005$ ; Fig. 2). Hens in Series 2 spent on average  $381.6 \pm 231.0$  s ( $N = 16$ ) in front of the familiar and  $217.3 \pm 153.5$  s ( $N = 16$ ) in front of the unfamiliar hen. This difference was statistically not significant, but may be seen as a strong tendency in the hens to stay longer with the familiar stimulus bird (paired *t*-test:  $t = 1.991$ ,  $N = 16$ ,  $P = 0.065$ ; Fig. 2).

To evaluate the influence of dominance on the preference behaviour, two indirect measurements of dominance were taken and analysed: body weight and comb size. The two measures strongly correlated with each other ( $r = 0.492$ ,  $N = 36$ ,  $F_{35} = 2.937$ ,  $P < 0.01$ ), but neither correlated with the aggregation time in front of the familiar nor the unfamiliar bird, neither in Series 1 nor in Series 2 (Series 1: weight:  $r = -0.030$ ,  $F_{19} = 1.062$ , NS and  $r = -0.355$ ,  $F_{19} = 2.101$ , NS, respectively; comb size:  $r = -0.369$ ,  $F_{19} = 2.170$ , NS and  $r = -0.336$ ,  $F_{19} = 2.012$ , NS, respectively; Series 2: weight:  $r = 0.373$ ,  $F_{15} = 2.190$ , NS and  $r = -0.316$ ,  $F_{15} = 1.924$ , NS, respectively; comb size:  $r = 0.177$ ,  $F_{15} = 1.430$ , NS and  $r = -0.396$ ,  $F_{15} = 2.311$ , NS, respectively).



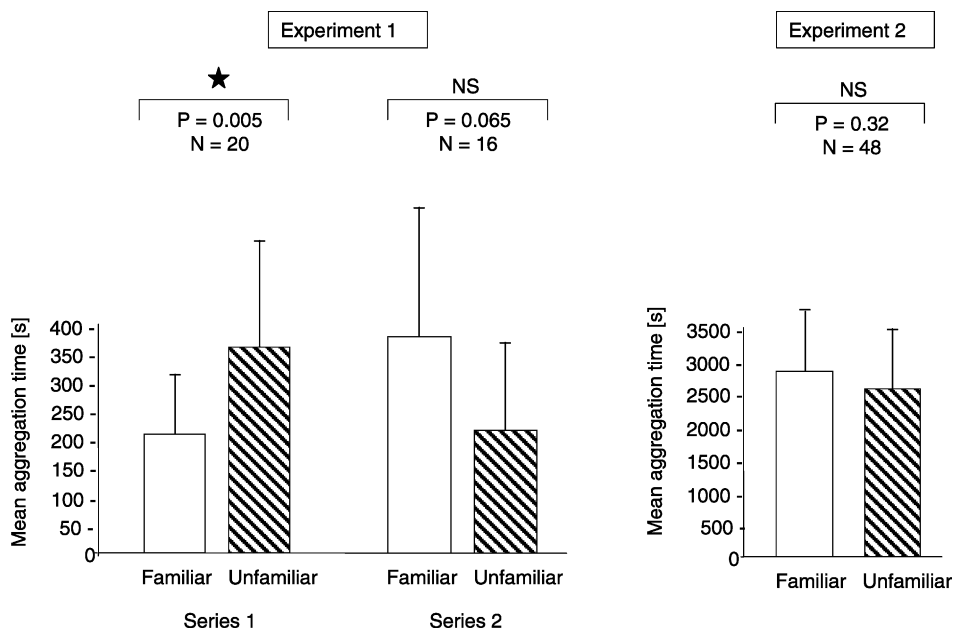


Fig. 2. Mean aggregation times with the familiar and unfamiliar stimulus hens of Experiment 1 (Series 1 and 2, observation time 20 min) and Experiment 2 (observation time: 90 min).

### 3.2. Experiment 2

In Experiment 2, the test hens could only rely on non-visual cues to distinguish between familiar and unfamiliar stimulus hens. In 11 out of 48 trials, hens exchanged calls during all eighteen 5 min intervals of the 90 min observation time. In 25 trials, calls occurred during 14–17 intervals, in nine trials during 8–13 intervals and in one trial during four intervals. In the course of the trial, calling activity decreased slightly but significantly (page test:  $L = 77421.3$ ,  $N = 48$ ,  $k = 18$ ,  $P < 0.05$ ). During the first two 5 min intervals, calls occurred in 100% of the trials, and during the last two intervals calls occurred in 70% of the trials. Different types of calls were given, which were not analysed further, but they were usually low and occurring more often when hens showed activity or just before they moved.

The hens spent significantly less time than expected within 25 cm of the dividing walls ( $\chi = 477.54$ ,  $P < 0.001$ ,  $N = 48$ ). The hens spent on average  $2828.08 \pm 893.44$  s ( $N = 48$ ) in front of the familiar and  $2569.75 \pm 892.85$  s ( $N = 48$ ) in front of the unfamiliar stimulus hen. This difference was statistically not significant (paired  $t$ -test:  $t = 1.002$ ,  $N = 48$ ,  $P = 0.32$ ; Fig. 2).

The influence of fearfulness on preference behaviour was assessed by the duration of the tonic immobility reaction. The mean duration of all test hens was  $435.5 \pm 315.3$  s ( $N = 46$ ) and did not correlate with aggregation time in front of the familiar or the unfamiliar stimulus bird (familiar:  $r < 0.01$ ,  $F_{44} < 1.2$ , NS; unfamiliar:  $r = 0.124$ ,  $F_{43} = 1.283$ , NS).

To be able to better evaluate the non-significant result of this preference test, a power analysis was carried out. The effect size in Experiment 2 (0.145) was about six times smaller than in Experiment 1 (Series 1: 0.733; Series 2: 0.886). Even though we increased the sample size from 20, respectively, 16 in Experiment 1, to 48 in experiment 2, the power decreased from 61.6% (Series 1) and 67.1% (Series 2) to 16.6% in Experiment 2. If the effect size in Experiment 2 had been the same as in Experiment 1, the power would have been more than 90%. To achieve a power of similar strength as in Experiment 1, a sample size of about 257 animals would have been needed. The minimal effect size, that we could have detected with our set-up ( $N = 48$ ) and with a reasonable power (80%) would have been 0.406.

#### **4. Discussion**

The preference test in Experiment 1 showed that domestic chickens do discriminate between familiar and unfamiliar conspecifics in an experimental situation where they were separated from the stimulus birds only by wire-mesh and therefore were able to use visual as well as auditory and olfactory cues. The fact that the hens in both series of Experiment 1 spent significantly more time than expected within 25 cm of the stimulus hens, suggests a general motivation for social contact. However, in the absence of visual cues (Experiment 2), they no longer discriminated significantly between familiar and unfamiliar birds, even though auditory communication could be observed over the whole 90 min intervals of the trials. The apparent subordinate role of non-visual recognition in the tested situation is underlined by the small effect size when compared to the situation with auditory, olfactory and visual cues altogether (Experiment 1). In situations where hens cannot see each other, familiarity does not seem to be the key point in deciding where they go. One reason for this might be that in natural situations, with only auditory and probably olfactory but no visual contact, it might be sufficient for a hen to know just where other hens are and not necessarily to know which individual they are. More distinct discrimination would then only be necessary in cases of direct encounters. From our results in Experiment 2 we may conclude that hens do not discriminate between familiar and unfamiliar conspecifics in the absence of visual cues, but this is not to say that they are unable to do so. It could be that they recognise other hens from their calls, but were not motivated enough to move closer. For future work, we suggest to test the hens in situations where they are highly motivated to distinguish between familiar and unfamiliar conspecifics, e.g. in an operant conditioning test or in situations with the choice of encountering or avoiding highly dominant birds. This would make it easier in case of non-significant results to decide whether the hens are not able or not willing to discriminate. What animals apparently prefer or not varies with the method of testing and even within individual animals (Dawkins, 1980). In other situations than the tested one, non-visual discrimination may be important. Additionally, there may be sexual differences in intra- and inter-sexual discrimination of conspecifics. Even if females would not discriminate between other females in the absence of visual cues, they may still discriminate between different cocks, which would biologically be meaningful. For cocks, on the other hand, non-visual discrimination between birds of the same sex could be important, but not of the opposite sex. For males, other males are concurrents whereas females are largely welcome, independent of their group identity.

In both series of Experiment 1, hens discriminated between familiar and unfamiliar birds, but their choice was not consistent. While they spent significantly more time in front of the unfamiliar stimulus bird in Series 1, they showed a strong tendency to aggregate longer in front of the familiar bird in Series 2. Keeping in mind that fowl are highly social birds, one would expect them to prefer the vicinity of a familiar bird to an unfamiliar one and this was confirmed in several studies (Stamp Dawkins, 1982; Bradshaw, 1992; Grigor et al., 1995; Stamp Dawkins, 1995; D'Eath and Stone, 1999; Marin et al., 2001). No references were found to preferences of unfamiliar conspecifics. Nevertheless, hens in Series 1 preferred unfamiliar birds and only hens in Series 2 showed a tendency to prefer familiar ones. We, therefore, looked for possible differences between the two series that could explain the opposite preference behaviour. We considered age, time of day, dominance and habituation.

Series 2 was conducted after Series 1 and birds were therefore older, but the two series were carried out within 12 days, starting at 16 weeks. At this age social hierarchy is already established, and the start of lay is still several weeks away, so that hormonal changes during the course of testing may be excluded. Trials of Series 1 took place between 09:00 and 13:00 h and trials of Series 2 between 09:00 and 16:00 h. The mean time of day when trials were conducted was significantly different between Series 1 and 2 (mean  $10:30 \pm 0.04$  h and  $12:26 \pm 0.09$  h, respectively), but had no effect on preference behaviour within a series. There was also no difference between birds in the two series in respect to dominance status as measured by comb size and body weight. The most plausible reason for the opposite results obtained in Series 1 and 2 are differences in the degree to which birds were habituated to the test box. While in Series 1 only naive test hens were used, test hens in Series 2 had additional experience with the test box because they had been used once as stimulus bird before they were used as test hens. When we compare the above mentioned studies which had found a preference for familiar birds with our study, especially Series 1, we realise that sample sizes were generally much smaller and, interestingly, that in all studies (except Marin et al., 2001) hens were more habituated to the test situation through additional training periods, longer initial habituation or repeated trials. We do not know more about how habituation may influence the decision to stay longer with a familiar or unfamiliar bird, but it is known that previous experiences may significantly influence the preference behaviour of hens (Dawkins, 1976), and therefore, habituation and its effect on the direction of preference behaviour in relation to familiar and unfamiliar conspecific may be an interesting and promising subject for further studies.

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