



## Research report

Breed-specific companions—Inter-individual distances reflect isolating mechanisms within domesticated chickens (*Gallus gallus* f.d.)Inga Tiemann<sup>a,b,\*</sup>, Gerd Rehkämper<sup>a</sup><sup>a</sup> Study Group Behaviour and Brain, C. & O. Vogt Institute of Brain Research, University of Duesseldorf, Germany<sup>b</sup> Scientific Poultry Yard of the German Association of Poultry Breeders, Bruno-Duerigen-Institute, Rommerskirchen, Germany

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## ABSTRACT

White Crested Polish (WCP) chickens are an interesting breed because of skull anatomy (crest), brain size and composition. This makes them attractive to investigate processes of selection that could parallel a step towards speciation in terms of ethological isolation. Lohmann Brown Classic (BL) and Red Leghorn (RL) were selected as comparative breeds to detect whether WCPs flock together as shown by shorter inter-individual distances within WCP than across breeds. WCP and BL were observed in the first year whereas RL served as comparative breed to WCP in the second year. Eggs of both breeds of each year were incubated at the same time, and chicks hatched and were raised together. Three young hens of each breed were randomly chosen and observed weekly in an open field situation for 20 min between the first and 31st week of life. Intra-breed distances differed significantly from those distances measured across breeds. Results demonstrate breed-specific flocking within observed breeds. This flocking behaviour may reflect breed-specific social and sexual preferences. Our observations indicate that domestic breeds may represent an ethological entity. Selective processes controlled by human intervention as given in domestication may therefore to be set in parallel to evolutionary processes.

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## 1. Introduction

The process of evolution results in the establishment of new species (speciation). Species are freely and fertile inbreeding groups which are reproductively isolated from other such groups (biological species concept BSC, [25]). This definition of species can be seen as a basic concept which was revised e.g. by Maynard Smith [24], who accepted the rare but not general existence of fertile hybrids without giving up the species level of their parents. One possible mechanism of the alteration of allele frequency according to the process of speciation is based on female choice [1]. The preference for a sexual mate can be seen as part of social behaviour, which, in birds, is developed from the time of hatching to adulthood and sexual maturity [13]. However, modern evolutionary theory is based on heritable traits [8]. Heritable traits can be involved in mate choice as shown by Fisher [10] and Lande [22]. Thus, the evolutionary process of speciation as driven by social and sexual mate preference should be based on heritable behaviour and

any reduction of gene flow between two populations. It is unknown whether such a process takes place under the general conditions of domestication.

Whether speciation as defined above occurs under the conditions of domestication is still under discussion. Some authors think not [18] others think so [28], their point of view on reproductive isolation and ecological niche affecting their attitude on this question. Opponents lean on the assumption that domestic chickens show sexual affinity to the wild-type and therefore would constitute a joint reproductive group. Proponents refer to adaptation to the anthropogenic environment and the history of selection which includes major alterations of alleles in the domesticated population. No experimental analysis has yet considered social and sexual mate choice in domesticated animals.

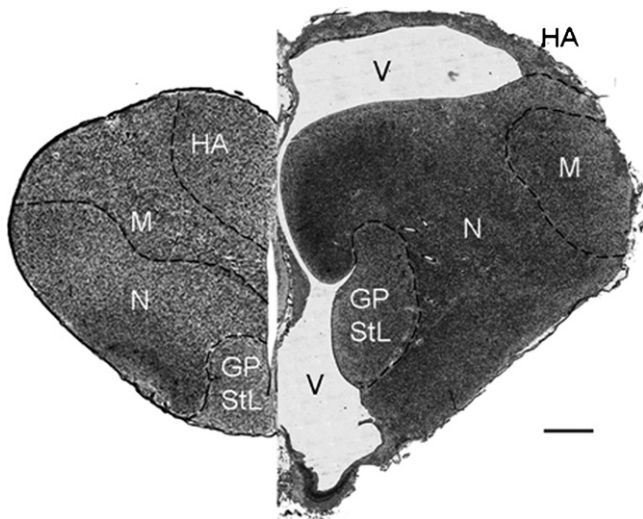
We have investigated social mating preferences of domestic chicken breeds in terms of possible underlying preferences which may influence inter-individual distances.

White Crested Polish (WCP) chickens were chosen for this investigation. They represent an old, non-commercial breed belonging to the group of crested chickens that can be traced back approximately 1600 years [4]. Krautwald [21] and others described the characteristic crest and head anatomy of crested chickens.

Based on morphometric studies on serial sectioned brains, Frahm and Rehkämper [11] and Rehkämper et al. [29] demon-

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**Fig. 1.** Coronal section through the brain of a Japanese Bantam (left) and a White Crested Polish chicken (right) to illustrate some subdivisions. HA, Hyperpallium apicale; M, Mesopallium; N, Nidopallium; GP, Globus pallidus; StL, Striatum laterale; V, Ventricle. Gallyas stain for cell bodies [15], scale 1 mm.

strated that the brains of WCP individuals are significantly larger than in other breeds and are characterized by a unique brain composition. Compared with other domestic chicken breeds WCP represent a separated and self-contained population in this investigation. The diencephalon and the telencephalon were significantly enlarged among White Crested Polish chickens compared to Bantams (Japanese Bantams, Peking Bantams), Mediterranean chickens (Brown Leghorns), chickens originally bred as game birds (Malayans), another crested chicken breed (Breda) and those with peculiar external characters such as tailless Araucanas and Silky chickens with silky feathers [29]. The enlargement of the telencephalon was mainly caused by an enlargement of the nidopallium and the mesopallium in WCP compared with Japanese Bantam [Fig. 1; 11] and other chicken breeds. Parts of the hyperpallium, although not obvious in this section, are also significantly enlarged [11]. The increases are independent of the enlarged ventricles in the WCP. These mentioned regions are known to be responsible for learning and cognitive abilities in general [26]. All of these peculiarities make WCP outstanding among domestic chickens and thus suitable to test whether speciation might occur under domestication.

In the present study the occurrence and establishment of social mate preferences of WCP were investigated during ontogenesis in comparison to a commercial breed, Lohmann Brown Classic (BL) and a pure breed, the Red Leghorn (RL). These breeds were chosen because their genetic variability is low and because they represent two different colours which might play a role in social/sexual behaviour [23,32]. The history of both breeds is comparatively long since the ancestors of both the Mediterranean Leghorns were firstly described approximately 2000 years ago. It has been observed that coloured chickens are sexually more attractive than white ones [23]. Whereas BL show a brown colour the plumage of the RL is similar to the wild-type, the plumage of the Red Jungle Fowl. It has already been discussed that the question of sexual affinity is a crucial one in context of the discussion on speciation. Additionally both breeds are of a size similar to WCPs.

As a measure of social preference we have chosen the inter-individual distance because preliminary field studies (not published) have suggested that this might be a valuable criterion that is quantifiable. There are data from other investigations that support this (Arnold and Maller [2] in sheep; Keeling and Duncan

[20] and Mench and Keeling [27] in chicken). To reduce imprinting and learning effects to a minimum all chicks of WCP and the breed of comparison were raised together in the present study. Thus, they shared all experiences; especially during the time of social learning that ranges from 4 days before hatching to 16 weeks of life [19,33].

## 2. Methods

### 2.1. Breeds

Experiments were carried out over two consecutive years. In the first year, eggs of WCP and BL chickens were incubated and hatched. In the second year, WCP and RL chickens were observed. Eggs of WCP and RL were received from private breeders, who breed their animals according to the 'Rassegefügelstandard für Europa' (European Standard of Perfection in Poultry Breeding) which verbalises objectives of morphology [5]. The quality of these breeders is proved by taking part successfully in national championships. The eggs of the commercial strain of Lohmann chickens were bought from a local trader.

In both years, eggs of WCP and eggs of the comparative breed were incubated so that the chickens hatched within 1 week. After being kept in small brooding boxes for 5 days all chickens of both breeds were raised together in a chicken house (8 qm<sup>2</sup>) with a bordering run of 250 qm<sup>2</sup>. In total, 50 individuals per breed were kept in the beginning of the observation period. All cocks but seven per breed were removed from the group of additional 25 females per breed respectively 50 females in total as soon as their sex was identifiable. These 14 cocks were in the group at the time of the study but took no part in the experiment itself. Chicks were fed ad libitum and were not part of any other studies for the time of observation. All chickens were experimentally naïve and shared the same experiences. Because of the experimental design, observations of three hens per breed in a group of six hens in total were made once a week. It was ensured that every individual, which was chosen randomly for each composition of this group, was observed as often as other individuals respectively for the same amount of time by the end of the observation period. This period ranged from the 1st to the 31st week of life and lead to a total of 93 observations per breed per year. The results of the 10th and 11th week of life with WCP and BL are missing because of a computer error.

The handling of the animals and the protocol of the experiment were the same in both years. After capturing the hens, they were identified by leg bands, transported in a wooden box and placed into an open field. Individuals of different breeds were placed alternately into the open field. The open field was located in a room that was separated from their living space. It had a floor that was painted grey with a colour that allowed easy cleaning. The borderlines of the open field measured 190 cm × 190 cm and they were bounded by a wooden frame of 80 cm height.

### 2.2. Observations

The experimental groups were observed in the open field for 20 min, after the experimenter left the room. The behaviour of the animals was filmed with a security camera and recorded using a DVD-recorder. Recordings were analysed with the program Viewer (Bioobserve GmbH, Bonn, Germany) on a computer. The relevant data were the distances between the chickens. The affiliation of individuals to a breed category was done by automatic phenotype classification. The distances seen on the computer screen were calibrated to those of the open field and were measured throughout the observation with a frequency of 10 frames per second. There were three intra-breed distances per breed that means between WCPs and between BLs or RLs. Also there were nine distances across breeds that are between WCP and BL or between WCP and RL.

### 2.3. Statistical analyses

Distances among and across breeds were given by the computerized calculation of the social contact module of the program Viewer. The Friedman Repeated Measures Analysis of Variance on Ranks was used to identify a general significance of the raw data set of each year because data were not normally (Gaussian) distributed. Medians + $Q_3$  (75th percentile) and - $Q_1$  (25th percentile) respectively are shown in graphs. To compare the distances by groups a Pairwise Multiple Comparison Procedures (Tukey Test) was used.

## 3. Results

The results of the first year for WCP chickens and BL are shown in Fig. 2. Significant differences in this data set were indicated by the Friedman RM ANOVA on Ranks ( $\chi^2 = 14,966$  with 2 d.f.,  $P = <0.001$ ). The distances among WCP and among BL are similar (RM ANOVA Tukey Test,  $q = 0.186$ ;  $N = 29$ ;  $P \geq 0.05$ ). Detailed values are summarized in Table 1. Distances across breeds differ significantly from distances among BL, whereas the last-mentioned were shorter (RM

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