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# Laying hens tweet. They also lead and follow! Implications for genetic selection against gregarious nesting

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#### Abstract

Gregarious nesting occurs when a hen chooses a nest already occupied while other nests are vacant, increasing welfare and production problems. This study aims to propose two predictors of gregarious nesting: weighted indegree (WID) and weighted outdegree (WOD), which are the number of times a hen entered an individual nest next to a vacant nest or an occupied nest, respectively. Nesting behaviour was recorded using individual electronic nests for 210 days. Genetic parameters for WID, WOD and nesting behaviour traits were estimated for 1,268 and 1,080 Rhode Island Red and White Leghorn respectively. For both lines, heritability estimates were high for WID (0.40/0.53) and low for WOD (0.12/0.15). Moderate genetic correlations with some nesting behaviour traits. WID and WOD could help to select against gregarious nesting but the effects for hens using collective nests remain to be evaluated.

#### Introduction

In the EU, the majority of laying hens are now raised in cage-free systems, such as barn, freerange, and organic (52% in 2020; European Commission, 2021). In these systems, hens lay their eggs in collective nests, allowing them to express natural nesting behaviours (Cronin et al., 2012). Gregarious nesting can occur when a hen chooses a nest already occupied while other nests are vacant (Riber, 2010). This behaviour can lead to mortality with suffocation in the nest and the increase of broken eggs and floor eggs (van den Oever et al., 2021). Selecting hens against gregarious nesting could help to reduce welfare problems and to increase egg production in cage-free systems. However, this behaviour is difficult to record on large scale. Currently, little is known about its genetic background. The development of electronic nests continuously recording the nesting behaviour of hens raised in groups, could make it possible to identify predictors of gregarious nesting (Bécot et al., 2021a). This study aims to propose predictors of gregarious nesting, estimate their heritability and their genetic correlations with nesting behaviour traits.

#### **Materials & Methods**

**Birds.** Two laying hens' pure lines – Rhode Island Red (RIR) and White Leghorn (WL) – belonging to the NOVOGEN company (Plédran, France) were mobilised. For RIR, the phenotypes were recorded between 2018 and 2021 on 1,268 hens (three batches) issued from 144 sires and 506 dams. For WL, the phenotypes were recorded between 2019 and 2021 on 1,080 hens (two batches) issued from 100 sires and 372 dams. For both lines, more than 5 generations were used in the pedigree to estimate genetic parameters. For each line and batch, birds were raised in a barn on the floor until 17 weeks of age. Birds were then transferred and raised in another barn until 64 weeks of age. This barn was composed of floor pens (one by line) and was equipped with individual electronic nests overlaid on two rows (about 1 nest for 5 hens). Hens were raised with cocks (about 1 cock for 10 hens).

**Recording system.** Nesting behaviour was recorded continuously by radio-frequency identification and using individual electronic nests developed by NOVOGEN. Hens were individually recognised when they visited a nest by a transponder tagged on their leg. Thus, the hen's identification, the localisation of the nest visited, the time of entry in the nest, and the time of exit from the nest were recorded for each visit. Oviposition time was recorded with an egg sensor located behind each nest, allowing us to know if the hen had laid an egg. Data were recorded between 200 and 409 days of age. Thus, 233,127 and 198,760 visits with oviposition were collected for RIR and WL, respectively.

*Traits.* The recording period was divided into seven 30-days periods. The pairs of hens using a nest for laying at the same time, in nests separated by a horizontal distance of 0 or 1 nest (0 for those at the same horizontal position, on top and bottom row) were selected to calculate predictors of gregarious nesting. This represented on average 2,617 and 3,486 pairs by day for RIR and WL respectively. For each hen of the pairs, the first who was entered in the nests was considered as a leader and the second as a follower. Using this information, two social network traits were calculated by hen as predictors of gregarious nesting: weighted indegree (WID) and weighted outdegree (WOD; Bondy and Murty, 1976). WID was the number of times the hen was the leader. WOD was the number of times the hen was the follower. Finally, hens with at least 15 eggs laid in the nests for a given 30-days period were kept for analyses, as hens with fewer eggs had fewer opportunities to express gregarious nesting. Thus, 8,137 and 7,124 phenotypes respectively associated with 1,268 RIR and 1,080 WL were kept for analyses (Table 1).

	Rhode Island Red			White Leghorn				
Trait	Mean	Std	Min	Max	Mean	Std	Min	Max
WID	64.13	40.63	1.00	317.00	92.45	47.73	5.00	425.00
WOD	64.36	24.53	0.00	134.00	92.60	24.76	2.00	137.00
LRN (%)	94.57	7.56	50.00	100.00	92.11	8.33	50.00	100.00
MOT (hh:mm)	02:47	01:23	-00:23 <sup>a</sup>	08:12	03:36	01:05	-00:12 <sup>a</sup>	07:31
LVOT (sq. h)	-0.62	1.42	-6.28	3.29	-0.15	1.25	-4.14	3.54
MDN (nests)	7.70	4.09	0.12	31.50	10.55	4.64	1.48	33.17
MLD (min)	41.23	18.99	6.54	123.80	58.75	27.99	9.17	193

Table 1. Summary statistics of phenotypic data.

WID, weighted indegree; WOD, weighted outdegree; LRN, laying rate in the nests; MOT, mean oviposition time; LVOT, logarithm of the variance of oviposition time; MDN, mean distance between nests of laying; MLD, mean laying duration. <sup>a</sup> MOT before the lights were turned on.

Five nesting behaviour traits were then calculated for each of these hens and each 30-days periods. The laying rate in the nests (LRN) was calculated to represent the egg production in the nests. For a given 30-days period, LRN was the egg number laid in the nests divided by 30 and then multiplied by 100. The mean oviposition time (MOT) and the logarithm of the variance of oviposition time (LVOT) were calculated to characterize the laying rhythm, using a procedure described in Bécot et al. (2021b). To consider the pre-laying behaviour referring to the nest choice for laying, the mean distance between nests of laying (MDN) was calculated. The distance between nests of laying corresponded to the horizontal distance, in the number of nests, which separated the nests of laying for two consecutive days. To avoid measuring different behaviours, this distance was not calculated for the laying separated by one or more days without laying in the nests. The mean laying duration (MLD) was the mean of the time spent in the nest for visits with oviposition. The time spent in the nest was calculated by the

difference between the time of exit from the nest and the time of entry in the nest. The summary statistics of the 7 traits are given in Table 1. All traits were standardized to facilitate the convergence of models.

*Model.* Genetic parameters and phenotypic correlations were estimated using the restricted maximum likelihood method applied to a multi-trait animal model. The environmental effects of the model included the fixed effect of the hen's hatch date and the random effect of the common environment of the hen. Variance and covariance components were estimated for the 7 traits together with REMLF90 (Misztal et al., 2002). The estimated values were used as starting points to estimate standards errors with AIREMLF90 (Misztal et al., 2002).

#### **Results and discussion**

*Heritability estimates.* Heritability estimates for WID and WOD were high (0.40) and low (0.12) for RIR respectively (Table 2). Similar results were found for WL with heritability estimates from 0.53 and 0.15 for WID and WOD respectively (Table 3). To our knowledge, no heritability estimates for these traits were reported in the literature. However, van den Oever et al. (2021) observed a different expression of gregarious nesting (considering the number of eggs per nest) between five broiler breeders lines and suggested the influence of genetic background.

Table 2. Genetic	parameters and	phenotypic	correlations <sup>a</sup>	for Rhode	Island Red.
	1				

Trait	$h^2$	$r_g / r_p$ WID	$r_g / r_p$ WOD
WID	0.40 (0.05)	1 / 1	
WOD	0.12 (0.03)	0.26 (0.15) / 0.33	1 / 1
LRN	0.07 (0.02)	0.02 (0.28) / 0.21	0.69 (0.42) / 0.37
MOT	0.48 (0.06)	-0.61 (0.07) / -0.55	-0.13 (0.15) / -0.22
LVOT	0.19 (0.03)	-0.51 (0.10) / -0.34	-0.36 (0.15) / -0.30
MDN	0.19 (0.04)	0.14 (0.14) / 0.15	0.20 (0.19) / 0.11
MLD	0.46 (0.06)	0.78 (0.05) / 0.66	0.02 (0.16) / 0.00

Traits abbreviations are described in Table 1. <sup>a</sup> Heritability estimates ( $h^2$ ) for all traits. Genetic ( $r_g$ ) and phenotypic ( $r_p$ ) correlations between predictors of gregarious nesting (WID and WOD) and nesting behaviour traits. Standard errors are in parenthesis.

*Genetic correlations.* Moderate genetic correlations, from +0.26 and -0.35, were estimated between WID and WOD for RIR and WL respectively (Table 2 and 3). Counterintuitively, WID and WOD are not genetically opposite and would provide different information linked to gregarious nesting.

For RIR, strong genetic correlations were estimated between WID and the traits MOT (-0.61), LVOT (-0.51) and MLD (+0.78). For WL, strong genetic correlations were estimated between WID and MLD (+0.87). For both lines, these results suggest selecting the leader hens, who have a high breeding value for WID, would increase MLD. This is not desirable as high MLD increases the occupation rate of the nests and therefore the risks for some hens to lay floor eggs (Bécot et al., 2021a).

Strong genetic correlations were estimated between WOD and LRN (+0.69; but with a high standard error) for RIR or between WOD and MOT (+0.43) for WL. This suggests selecting the not follower hens, who have a low breeding value for WOD, would decrease LRN and increase MOT for RIR and WL respectively.

For both lines, low genetic correlations, ranging from -0.08 and +0.14, were estimated between MDN and the traits WID and WOD, except between WOD and MDN for RIR (+0.20). Some

studies suggest that hens laying eggs in the same nests, i.e. with a low MDN, have a gregarious nesting (Riber, 2010; van den Oever et al., 2021). MDN would be complementary to WID and WOD, each of them capturing a different part of the genetic variance for gregarious nesting. Selecting the leader and the not follower hens, as well as hens laying their eggs in nests far from each other (high breeding value for MDN), could help to reduce gregarious nesting. However, this behaviour appears complex because it is influenced by multiple factors currently poorly known (Riber, 2012). The strong and unfavourable genetic correlations for WID and WOD with some nesting behaviour traits suggest to be careful before use such traits in breeding programmes. The real response to selection of WID and WOD to reduce gregarious nesting must be evaluated.

Trait	$h^2$	$r_g / r_p$ WID	$r_g / r_p$ WOD
WID	0.53 (0.06)	1 / 1	
WOD	0.15 (0.04)	-0.35 (0.15) / 0.06	1 / 1
LRN	0.09 (0.03)	-0.18 (0.25) / 0.22	0.36 (0.23) / 0.47
MOT	0.39 (0.06)	-0.33 (0.11) / -0.38	0.43 (0.14) / 0.37
LVOT	0.21 (0.04)	0.02 (0.13) / -0.18	-0.22 (0.16) / -0.31
MDN	0.24 (0.05)	0.14 (0.14) / 0.04	-0.08 (0.18) / -0.05
MLD	0.70 (0.07)	0.87 (0.03) / 0.73	-0.38 (0.14) / -0.11

Table 3. Genetic parameters and phenotypic correlations<sup>a</sup> for White Leghorn.

Traits abbreviations are described in Table 1. <sup>a</sup> Heritability estimates ( $h^2$ ) for all traits. Genetic ( $r_g$ ) and phenotypic ( $r_p$ ) correlations between predictors of gregarious nesting (WID and WOD) and nesting behaviour traits. Standard errors are in parenthesis.

#### Conclusion

This study highlighted that WID and WOD are heritable traits in a RIR and a WL lines. Such traits could be interesting to reduce gregarious nesting by selection. However, strong and unfavourable genetic correlations were estimated with MLD and LRN, according to the trait and the line. Some studies are needed to better understand the potential effects for selection of WID and WOD, especially consequences on gregarious nesting of commercial hens raised with collective nests.

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