



Genetic and phenotypic profiles of hens that pile

Final Project Report | JUNE 2025

A report for Australian Eggs Limited

by P.S. Taylor, A. Johnny, S. Mutch, R. Turnbull, A. Kashev,
M. Fogarty and M.J. Toscano

Australian Eggs Limited Publication No. INNO021

© 2026 Australian Eggs Limited.
All rights reserved.

ISBN 978-1-920835-94-1

Project Title: Genotypic and phenotypic profiles of hens that pile

Australian Eggs Limited Project Number: INNO021

The views expressed and the conclusions reached in this publication are those of the author and not necessarily those of persons consulted. Australian Eggs Limited shall not be responsible in any way whatsoever to any person who relies in whole or in part on the contents of this report.

This publication is copyright. However, Australian Eggs Limited encourages wide dissemination of its research, providing that it is clearly acknowledged. For any other enquiries concerning reproduction, contact the Sustainability Program Manager on 02 9409 6999.

Researcher/Author Contact Details

Name: Peta Taylor
Address: Faculty of Science, School of Agriculture, Food and Ecosystem Science
The University of Melbourne
Parkville, Victoria 3010
Phone: +61 3 8344 9102
Email: peta.taylor@unimelb.edu.au

In submitting this report, the researcher has agreed to Australian Eggs Limited publishing this material in its edited form.

Australian Eggs Limited Contact Details:

Australian Eggs Limited
A.B.N: 66 102 859 585
Suite 7.01, Level 7, 1 Pacific Highway
North Sydney NSW 2060

Phone: 02 9409 6999
Fax: 02 9954 3133
Email: research@australianeggs.org
Website: www.australianeggs.org.au

Published in July 2026

Foreword

This project investigated whether the propensity to pile was related to hens' genotype and/or behavioural phenotype. Piling is an abnormal behaviour where hens mass together, often on top of each other, which can lead to smothering. Smothering refers to a hen's death from suffocation. It can occur as hens pile in response to a fright (panic smothering), to access a specific nest box (nest box smothering), or for reasons unknown (recurring smothering). Previous research suggests that smothering accounts for 16% of mortality on free range farms in Australia.

This project was an international collaboration between researchers at the University of Melbourne and the University of Bern. Using radiofrequency identification technology across five pens in a semi-commercial barn, this project identified individual hen piling behaviour and the relationships with genetics, responses to novel stimuli and interactions with humans throughout the production cycle. Although not all piling leads to smothering, all smothering starts with piling. Therefore, understanding the underlying motivation for hens to pile is required to develop intervention strategies to reduce piling and smothering on laying hen farms.

This project was funded from industry revenue, matched by funds provided by the Australian Government and contributions from the Swiss Federal Food Safety and Veterinary Office and Open Philanthropy.

This report is an addition to Australian Eggs Limited's range of peer-reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

Most of our publications are available for viewing or downloading through our website:

www.australianeggs.org.au

Printed copies of this report are available for a nominal postage and handling fee and can be requested by phoning (02) 9409 6999 or emailing research@australianeggs.org.au.

Acknowledgments

The authors wish to thank and acknowledge the small, but efficient and precise, team that managed the manual video observations to pair and validate the RFID data, including Lara Fanning, Daniel Lienhard, Masha Marincek and Tatiana Zingre. We also thank Abdulsatar Abdel Rahman for the maintenance of the RFID technology and Aviforum staff for the animal care and management.

The authors are extremely grateful to the project steering committee for its feedback throughout the project.

Australian Eggs Limited, the Swiss Federal Food Safety and Veterinary Office and Open Philanthropy provided the funds that supported this project.

About the Authors

Peta Taylor is a Senior Animal Science lecturer in the School of Agriculture, Food and Ecosystem Sciences, and a poultry welfare scientist with the Animal Welfare Science Centre. Her research aims to improve the lives of chickens by providing evidence-based solutions, particularly around the welfare risks and opportunities associated with housing and management practices.

Michael Toscano is the group leader of the Centre for Proper Housing: Poultry and Rabbits (ZTHZ) at the University of Bern. Dr Toscano's research aims to improve hen welfare, particularly focused on hens in cage-free systems, and using technology to understand individual hen experience, behaviour and welfare.

Alex Johnny is a postdoctoral researcher at the ZTHZ at the University of Bern. His research focuses on understanding maladaptive behaviours such as piling and toe-pecking in loose-housed systems for hens.

Dr Alexander Kashev is a data scientist at the University of Bern, with extensive experience in developing data analytics to capture hen behaviour from radio frequency identification technology (RFID).

Robert Turnbull is a senior research data specialist at the Melbourne Data Analytics Platform (MDAP) at the University of Melbourne, where he specialises in Bayesian statistics and machine learning.

Simon Mutch is a senior research data specialist within the MDAP at the University of Melbourne, with expertise in high-performance computing, scientific modelling, data visualisation and working with large datasets.

Maxine Fogerty is a PhD student and Senior Research Assistant in the Animal Welfare Science Centre at the University of Melbourne. Maxine is investigating piling and smothering behaviour in a range of contexts.

Table of Contents

Foreword.....	iii
Acknowledgments.....	iv
About the Authors	iv
List of Tables	vi
List of Figures	vi
Abbreviations.....	viii
Executive Summary.....	ix
Overall Conclusions.....	x
1 Introduction	1
2 Materials and methods.....	3
2.1 Housing and animals.....	3
2.2 Timeline of events	3
2.2.1 Tracking behaviour.....	4
2.3 Detecting piling events	6
2.3.1 RFID data extraction and cleaning	7
2.4 Time spent on the litter	8
2.5 Assessing behavioural phenotype	8
2.5.1 Novel object test	8
2.6 Statistical analysis.....	9
2.6.1 Flock piling characteristics over time.....	9
2.6.2 Repeatability of piling behaviour	9
2.6.3 Bayesian inference	10
3 Results.....	12
3.1 Flock piling characteristics.....	12
3.2 Individual piling behaviour	18
3.2.1 Location immediately before a piling event	21
3.2.2 Genetic relationships with piling.....	23
3.2.3 Repeatability estimates of piling behaviour	24
3.2.4 Repeatability of hens piling irrespective of location.....	26
3.3 Novel object test	27
3.3.1 Repeatability estimates of novel object interactions	34
3.4 The relative effects of genotype and behavioural phenotype propensity to pile.....	34
4 Discussion.....	38
5 References	40
6 Plain English Summary	42

List of Tables

Table 1	A timeline of events reflecting the specific data collection on the flocks (pens) of hens involved in the project and events that were unrelated to this project (i.e. manual health checks) for context.....	3
Table 2	Conditions that must be met for the behaviour to be classified as a pile by trained observers.....	6
Table 3	Conditions that must be met for an individual hen to be classified as part of a piling by the trained observers	7
Table 4	The novel objects that were provided in each pen at each observational window	9
Table 5	The impact of sire, pen and individual hen on the likelihood that a hen would repeatedly join a pile	24
Table 6	The impact of location before a piling event (on the litter or not), and interaction between time point and location before a piling event on the likelihood that an individual hen would repeatedly join a pile.....	26

List of Figures

Figure 1	Schematic of the semi-commercial pen layout showing the side (top left) and top-down (top right) views – including aviary, scratch/litter and outside areas, and the location of the radio frequency identification antennas (pink and orange rectangles) to detect individual hen location.....	5
Figure 2	An image of the overlay editor that was developed by Dr Alexander Kashev	6
Figure 3	The number of piling events over three-day observation periods at 20, 30, 52 and 72 weeks of age for each pen	12
Figure 4	The estimated number of piling events at each time point based on a Poisson mixed model controlling for pen as a random factor	13
Figure 5	The duration of piles for each pen at each age	14
Figure 6	The estimated duration of a piling event at each time point from a GLMM gamma distribution, controlling for pen as a random factor	15
Figure 7	The estimated number of hens that participated in a piling event at each time point from a GLMM controlling for pen as a random factor	16
Figure 8	The estimated average entry fraction index for hens that participated in a piling event at each time point from a GLMM controlling for pen as a random factor	17
Figure 9	The estimated average exit duration fraction for hens that participated in a piling event at each time point from a GLMM with Beta distribution, controlling for pen as a random factor	18
Figure 10	The proportion of hens that participated in a piling event across four observation times at 20 (TP#1), 30 (TP#2), 52 (TP#3), and 72 (TP#4) weeks of age	19
Figure 11	The distribution of the number of piles that individual hens participated in	19
Figure 12	A density plot showing the distribution of the minimum (blue), maximum (dark blue), mean (orange) and median (red) time a hen spent in a piling event.....	20
Figure 13	Heat maps showing the order (rank) in which a hen entered and exited a piling event for each event (left) and the average rank for each hen across all piles she participated in (right)	20
Figure 14	The fraction of total pile duration at which a hen entered (0% start of pile; 100% end of pile) and the fraction of total pile duration at which a hen exited	21

Figure 15	The proportion of hens that participated in a pile (right) or not (left) that were located on the litter (orange bars) or not (blue bars) immediately before the pile started.....	21
Figure 16	The proportion of hens that were found on the litter immediately preceding a piling event (orange bars) or not (blue bars), and the propensity to join a pile (left) or not (right)	22
Figure 17	Comparisons of the odds ratio of a hen participating in a pile, if she was not on the litter (left) or was on the litter (right) immediately before a piling event at different time points	23
Figure 18	The number of piles (A), time spent in a pile (B), order of joining piles (C), and the participation fraction (D) for hens observed across four observation windows in the production cycle, relative to their sire	24
Figure 19	Predicted values for how long a hen spent in a pile as a function of time/weeks of age (WoA).....	25
Figure 20	Predicted latency for hens to join piles with age	26
Figure 21	Estimated marginal means of time spent with the novel object by an individual hen at four observation time points.....	27
Figure 22	Estimated marginal means of time taken to first approach the novel object (latency) for an individual hen at four observation time points	28
Figure 23	The total novel object (NO) interaction duration for each individual hen, split by sire.....	28
Figure 24	The total novel object (NO) interaction duration for individuals split by sire and time point	31
Figure 25	The total novel object interaction duration as a function of the number of piles (lasting longer than 20 minutes) each individual participated in.....	32
Figure 26	The total novel object interaction duration as a function of the number of piles (lasting longer than 20 minutes) each individual participated in, split by time point	33
Figure 27	The posterior predictive probability for the number of piles an individual participates in	34
Figure 28	The distribution of effect parameters from our Bayesian generative model	35
Figure 29	The expected log pointwise predictive density (ELPD) leave-one-out (LOO) scores for four different Bayesian models, which include or exclude the contribution of phenotype (novel object interaction) or genotype (sire) to piling behaviour	36
Figure 30	The effect of novel object interaction duration on piling behaviour as a function of interaction duration	36
Figure 31	The mean probability of a hen participating in a pile as a function of total time spent interacting with the novel object (NO) (dashed line), derived from the best model fit (i.e. without sire)	37

Abbreviations

CTAT	Caretaker approach test
HAT	Human approach test
ICC	Intraclass correlation
NO	Novel object
NOT	Novel object test
RFID	Radio frequency identification technology
WoA	Weeks of age

Executive Summary

Piling and smothering remain significant welfare concerns in cage-free laying hen systems. Smothering can account for up to 16–40% of flock mortality, often occurring suddenly and without clear external triggers. All smothering is preceded by piling, but not all piling results in smothering. Despite this, little is understood about the individual, genetic, or behavioural drivers of piling behaviour. This project investigated the individual behavioural drivers of piling in laying hens, a precursor to smothering and aimed to quantify individual variation in piling behaviour across the production cycle, and assess the roles of behavioural phenotype, age, and genetics.

Across four key production stages (20, 30, 52 and 72 weeks), over 1000 hens ($n = 225$ in each of 5 pens) were tracked using a combination of manual video observations and radio frequency identification (RFID) tracking technology to detect piling participation and responses to novel objects and an acute commercial stressor. Piling was frequent and intensified with age, including the frequency, duration and number of hens piling. Up to 50% of hens participated in a piling event, with a small, consistent minority (~10%) never piling. Early in the production cycles, hens that were already on the litter were over 3 times more likely to join a pile. However, this association diminished with age/experience, as older hens increasingly moved from the aviary to join piles, in which the spatial proximity was less influential than motivation or social reinforcement in later life.

Bayesian modelling revealed that the strongest predictor of a hen's propensity to pile was behavioural phenotype, specifically her interaction with novel objects (NOs) across the production cycle. GLMM repeatability models and Bayesian modelling indicated that genetic background (i.e. sire) accounted for only a small proportion (1%) of the variation in piling behaviour. However, the individual hen explained 10–15% of the variation in piling behaviour. Notably, imposed commercial stressors, including short-term water deprivation and vaccination, did not increase piling, suggesting that recurring piling behaviour is not driven by acute environmental stress. Contrary to some earlier studies linking fearfulness to smothering, this work indicates that exploratory traits, not neophobia, may underlie the propensity to pile.

Together, the results show that piling is a complex and partly learned group behaviour that becomes more prevalent and efficient with age. The use of individual tracking systems provides new opportunities for managing piling through behavioural profiling, precision tracking, and targeted environmental modifications. However, strategies to reduce piling must be targeted in a way that does not compromise positive welfare traits, such as reducing hen curiosity or exploration. These findings arise from a controlled, semi-commercial research environment, and should be validated across additional flocks and commercial systems before broad industry implementation.

Overall Conclusions

This study advances the understanding of piling in laying hens by demonstrating that it is a frequent behaviour that escalates with age and cumulative experience, varies greatly between individuals and is strongly associated with behavioural phenotype, but only weakly associated with genetics. By using RFID technology to monitor over 1000 individual hens across their production cycle, we identified consistent individual participation patterns and age/experience-related changes.

Individual hens showed stable tendencies to engage in or avoid piling, with a small but consistent subset never participating. Behavioural phenotype, particularly curiosity and interaction with novel objects, was the strongest predictor of piling behaviour. In contrast, genetic influence (sire line) explained only a small, but statistically significant, proportion of variation (1%). As hens aged, they joined more piles more readily and remained in them for longer durations, suggesting that piling may be reinforced through social experience or increased sensitivity to group cues.

Importantly, hens' location on the litter immediately before a piling event predicted participation early in the laying cycle but became less influential over time. Furthermore, the occurrence of piling was not significantly affected by commercial stressors, such as short-term water deprivation and vaccination, suggesting that recurring piling is unlikely to be caused by acute environmental stressors. Instead, intrinsic factors like temperament or social dynamics appear to play a more central role.

These findings highlight a crucial distinction between recurring piling and panic-driven smothering, and suggest that strategies to mitigate smothering must consider the specific type of piling involved. While hens showing higher engagement with novel stimuli were more likely to pile, curiosity is associated with positive welfare traits. Interventions aimed at reducing piles must therefore avoid suppressing curiosity.

Behavioural profiling, supported by automated tracking technologies, offers promising tools for early detection and mitigation of piling. Potential strategies include modifying shed environments to reduce visual cue amplification, introducing dispersal cues during the early stages of pile formation, and selectively managing hens with high piling propensities without compromising welfare.

Overall, this work contributes evidence that piling behaviour is shaped by a complex interplay of individual traits, age, and social dynamics. Effective management of piling and smothering in cage-free systems will require both individual and group-level approaches that respect the behavioural diversity of hens while prioritising welfare.

1 Introduction

Smothering is a global welfare concern for laying hens, characterised by dense clustering of hens, during which some individuals may die from asphyxiation (Mazocco et al. 2024). Estimates suggest that smothering causes 16% of mortality in free range commercial flocks in Australia (Chowdhury et al. 2024), a figure consistent with international reports, such as 16–40% of layer mortality in the UK (Bright & Johnson 2011). Reports from Belgian egg producers note an increase in smothering after transitioning to cage-free systems (Stadig et al. 2015). The increase in smothering behaviour observed in cage-free systems relative to cage systems may reflect a shift in the hens' motivation to participate in piling behaviour, which leads to smothering, but likely reflects the greater number of hens that can participate in the behaviour and subsequently increase the risk of a fatal outcome. With much of the world shifting to cage-free housing for layer hens, smothering is likely to continue to increase. Smothering events are often unexpected, unavoidable, and the impact of each event on hens is unpredictable; some events result in very few hen deaths, but have been shown to result in > 900 hens dying in a single event (Chowdhury et al. 2024). Despite the widespread issue of smothering, very little is known about why hens smother and what motivates the preceding piling behaviour.

All smothering is preceded by piling, but not all piling results in smothering (Winter et al. 2022a). Piling is described as the clustering of hens, which can occur either in the presence of a resource (e.g. a nest box or pop hole) or without an obvious external stimulus (Winter et al. 2022a). Piling events can last for up to 2 hours, occur frequently throughout the day and result in extreme hen densities – up to 187.93 hens/m², posing both a significant welfare risk and management challenge (Herbert et al. 2021). Gray et al. (2020) proposed four hypotheses for the underlying causes of piling and smothering:

1. attraction/aversion of specific stimuli
2. social behaviour
3. early life experience
4. maladaptive collective behaviour.

These causes can be interpreted through both proximate and ultimate frameworks, referring to immediate triggers, such as environmental stimuli, psychological state, or developmental stage of the hens, or evolutionary mechanisms, including domestication and the evolution of collective behaviours, respectively (Gray et al. 2020).

None of these hypotheses have been conclusively supported or rejected, but emerging evidence points to the behavioural phenotype of hens, particularly fearfulness and curiosity, having a key role. For example, Chowdhury et al. (2025) reported an increased smothering risk in commercial free range flocks where hens were more fearful (or less curious) of novelty and less fearful of humans. This discrepancy may arise from observer bias, or methodological issues such as flock-level assessments, the inability to determine causation or failure to categorise types of smothering and piling.

Bright and Johnson (2011) report three types of smothering:

1. panic smothering
2. nest box smothering
3. recurring (or creeping) smothering.

Recurring smothering is a particular concern as it can persist and propagate through the flock, is difficult to predict, and remains poorly understood (Mazocco et al. 2024). Therefore, future research must differentiate between smothering (and piling) types and explore how each relates to behavioural phenotypes and environmental conditions.

Understanding the individual motivation to pile is an essential first step in determining whether genetic or phenotypic traits drive the behaviour. However, tracking individual hens poses significant logistical challenges. Large-scale behavioural monitoring requires shed-specific technology and specialised expertise to ensure that data are both biologically relevant and manageable. However, recent developments at the University of Bern have shown that radio frequency identification (RFID) technology can be used to monitor individual piling behaviour (Rice et al. 2023; Stratmann & Toscano 2023). Although this technology continues to improve, current implementation relies on manual detection and location mapping of piling events to guide data extraction, limiting the amount of data that can be feasibly collected across a hen's entire production cycle.

Assessing the repeatability of piling behaviour over time provides insight into the consistency of individual responses – as such, this can provide insight into the impact of temperament, genes, environment and their interaction on piling behaviour. For example, developmental experiences during rearing could affect hens' attraction to, or avoidance of, certain stimuli, shape social tendencies or alter stress responsiveness in adulthood (Gray et al. 2020). Identifying consistent individual differences could enable targeted interventions: in the short-term, environmental modifications during rearing to reduce maladaptive social clustering; and in the longer term, selective breeding for genotypes associated with lower piling and smothering risk. The first step to understanding if piling behaviour is influenced by genotype and phenotype is to calculate the degree of repeatability of the behaviour across time, i.e. is a hen's participation in piles stable over time?

This project aimed to:

1. quantify individual hen piling behaviour throughout the production cycle
2. assess the repeatability of piling behaviour within an individual across time/age
3. explore genetic contributions to piling by assessing piling behaviour from specific sires
4. identify relationships between behavioural phenotype and an individual's propensity to pile.

We hypothesised that an individual hen's propensity to pile would be:

1. repeatable throughout the production cycle
2. heritable, i.e. associated with her genetic profile
3. associated with consistent responses to novelty (i.e. hen temperament).

Reducing the risk of piling and smothering will support improved animal welfare, enhance production efficiency, and maintain social licence for egg production.

2 Materials and methods

The use of animals in this study was conducted according to the cantonal and federal regulations for the ethical treatment of experimentally used animals and approved by the Bern Cantonal Veterinary Office (BE33/2023). The component involving humans in this study was conducted and approved by the Melbourne University Human Ethics Committee (2024-28458-48-3).

2.1 Housing and animals

A full description of the barn and radio frequency identification technology used in this experiment has been reported in Perinot et al. (2025). Briefly, five aviary pens (7 m × 2.3 m), each with an adjacent wintergarden (4.1 m × 2.3 m), housed 225 ISA brown hens per pen. Hens were reared in two groups in a large open barn and transferred to the production aviary pens at 16 weeks of age. Each pen contained two square peck blocks on both sides of the aviary in the scratch area. Wintergardens were outside of the shed, adjacent to the indoor pen and were covered by a roof, closed with mesh wire, and had a dirt floor and nipple drinkers, and were available most days between 10:00 and 16:00. Hens were housed at a stocking density of 7.2 hens/m², including the aviary areas. Caretakers checked the health and welfare of the hens twice daily. Pen production data, including egg production, floor eggs, feed consumption and mortality, were recorded daily. Hens were descendants of one of 40 sires. Between 4 and 6 hens from each sire were placed into each pen. Animal density was 8.1 hens/m² in the indoor areas, which was reduced when pop holes were open, providing hens with access to a wintergarden (9.32 m²).

2.2 Timeline of events

Table 1 details the timeline of events throughout the project, detailing how hens were managed and how data were collected throughout the production cycle.

Table 1 Timeline of experimental data collection and concurrent flock management activities (e.g. routine health checks) undertaken throughout the study, presented by week of age (WoA).

Age of hens (weeks of age)	Event	Data collection and behavioural observations
16 WoA	Placement	Pullets moved from the rearing shed to the production shed
20 WoA	Behaviour assessment: time point #1	Piling observations #1 Human approach test #1 Novel object test #1 – coloured stick
30 WoA	Health assessment Commercially relevant stressor	Body weight, feather score, and foot health scores – Water vaccination
52 WoA	Behaviour assessment: time point #2 Behaviour assessment: time point #3	Piling observations #2 Human approach test #2 Novel object test #2 – mirror Piling observations #3
72 WoA	Health assessment	Human approach test #3 Novel object test #3 – LED strip Body weight, feather score, and foot health scores Piling observations #4

2.2.1 Tracking behaviour

Individual hens were tracked using an RFID system (Gantner Pigeon Systems GmbH, Schruns, Austria), which has been validated in various settings, including in situ (see Gebhardt-Henrich et al. 2023). Antennas were placed throughout the aviary tiers and litter area, and external to the wintergarden pop holes (Figure 1). Antennas on the floor were covered by a thin board for protection. All antennas were connected to reading devices via a multiplexer (Moxa, New Taipei City 242, Taiwan) and a computer. Hens were tagged on both legs, one with a unique RFID transponder and another for visual identification.

The behaviour of the hens was collected for four different time points (20, 30, 52 and 74 weeks of age) using two cameras (Samsung SCO-2080R, IR, Samsung Techwin Co., Korea), which recorded the two different litter areas within the pen. A customised recording software (Multieye Hybrid Recorder Version 2.3.1.8, Artec Technologies AG, Diepholz, Germany) was used to set up the video recording.

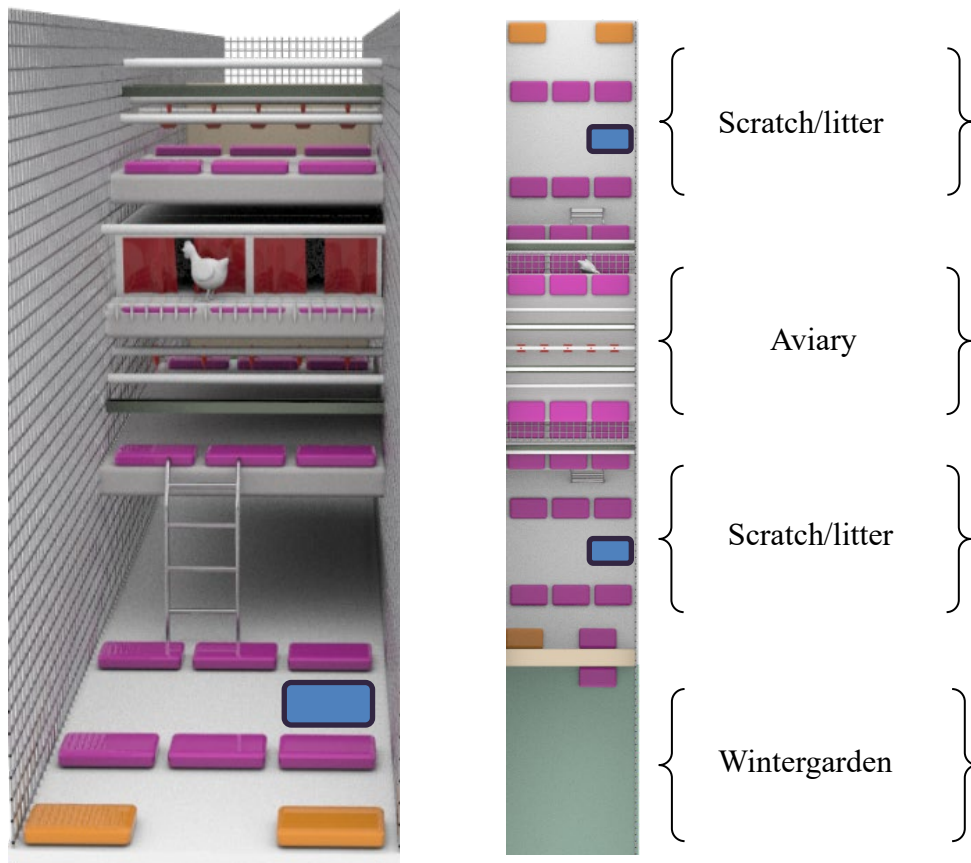


Figure 1 Schematic of the semi-commercial pen layout showing the side (top left) and top-down (top right) views – including aviary, scratch/litter and outside areas, and the location of the radio frequency identification antennas (pink and orange rectangles) to detect individual hen location

Blue antennas in the schematic indicate additional antennas funded by Australian Eggs, subsequently increasing the amount of litter area monitored.

The bottom image is an in-situ example of antenna placement on a scratch area (relevant for this project) before antennas were covered for protection and hens were tracked.

Each antenna contained 12 individual coils that permitted the collection of precise location information. Interactions with specific objects (i.e. novel objects) and the locations of piles were recorded at the individual coil level by the development of an overlay program by Dr Alexander Kashev (University of Bern) (Figure 2). The overlay editor tool required manually synchronising each antenna

and coil location for each camera. This information was then used to develop a camera-specific overlay superimposed on live videos to highlight the relevant antennas and coils of interest (i.e. those where a pile occurred).



Figure 2 An image of the overlay editor that was developed by Dr Alexander Kashev

The overlay editor was designed to input specific coordinates of antenna and coil locations for each camera across pens. Coordinates were used to develop an overlay for live videos. The overlay enabled manual observations of videos and recorded the location of the coils in the areas of interest (i.e. hens in a pile) and relevant RFID data.

2.3 Detecting piling events

Each pile was manually identified by analysing recorded videos from 05:00 to 16:00 for three days per time point, by one of three trained observers. A pile at the group level was defined as outlined in Table 2. The start of a pile was defined as ‘when all conditions 1–4 were met and continued for a minimum of 4 minutes – the start time was recorded from the first moment all conditions were true’. The end of a pile was defined as ‘when conditions 1–4 were not fulfilled for a minimum of 30 seconds. If more than one pile could occur at the same time (temporally overlapping piles at different locations, which were separated by a gap), they were treated as distinct piling events.

After a pile was identified, manual observations recorded the location of the pile and the RFID coils that were relevant, using an overlay tool. As piles were dynamic and often moved as hens pushed, joined or left the pile, the location (and therefore relevant coil information) was recorded every minute during the first 7 minutes of a pile, and every 2 minutes thereafter. The recording of coil locations of a pile started 30 seconds after the start of the pile (CR1)

Table 2 Conditions that must be met for the behaviour to be classified as a pile by trained observers

Pile conditions	Description
1	Seven or more hens at estimated maximum, though the pile starts/ends with 3 or more hens, i.e. 3 or more hens must always be in a pile.
2	Most hens were immobile, excluding head movement and movement caused by other hens (i.e. pushing in – see point 4).
3	Standing with body outlines overlapping; sitting hens were considered if they moved below two or more piling hens, or when they were sitting because they were inside a pile.
4	Most hens were facing the same direction or to the same spot along the sagittal axis. This is most applicable for smaller piles, e.g. three hens and defining the start of piles.

An individual hen was considered part of the pile when the criteria in Table 3 were met. The number of hens in a pile was the number of individuals that were part of the pile (Table 3; conditions 5–8). *Entry time to the pile was measured when conditions 5–8 were met, and exit time was considered when one of the conditions 5–8 was not met.*

Table 3 Conditions that must be met for an individual hen to be classified as part of a piling by the trained observers

Piling conditions	Description
5	A pile has started (start criteria above), and all five pile conditions are currently met, a hen can be assigned to a pile.
6	The individual hen was sitting or standing with overlapping body outlines (> 25 % of the body outline is covered) with other hens in the pile (only the neck or tail overlapping was excluded) for > 5 seconds.
7	The hen was mostly immobile. However, moving/not immobile hens were not necessarily excluded from the pile if they were moving (deeper) into the pile, moving only moderately with their head/neck, or getting pushed by other hens in a pile.
8	Absence of other functional behaviours, including foraging/pecking on the ground, preening/feather cleaning, dust bathing, aggression/pecking other hens, and floor nesting.

These recordings noted the specific coils of interest, as the pile changed in size or location. RFID data from the coils of interest (i.e. presence of hens on antennae sections where piling was occurring) were extracted within the 30-second window before and after the piling observation time point. For each pile, the pile characteristics were calculated, including duration of the pile, identity of participants, the latency of the individuals to join the pile, and the time spent by an individual in a pile. An individual was deemed to be a participant if it was detected at least once in the coils where piling was happening. For each pile, we generated a binary dataset where participants in the pile were assigned one, and all other hens allotted to that pen for that day were assigned zero. The latency to join the pile was defined as the difference between the first detection of an individual in a pile and CR1. For the latency to join the pile, all individual detections from the start of the pile to CR1 were assigned a latency of zero seconds. The time spent by an individual in a pile was defined as the difference between the first detection on coils outside the pile and the two neighbouring coils and CR1.

2.3.1 RFID data extraction and cleaning

Data extraction was conducted by Dr Alexander Kashev using R Studio in consultation with project researchers. Briefly, raw RFID files were read into R Studio, and every line was checked for consistency; any inconsistencies were discarded, and the remaining data were corrected for fractional seconds. Subsequently, the cleaned raw lines were parsed as a table merging additional columns based on Antenna ID and the timestamp, providing information on Pen, Side, Position, Location, orientation of the antenna (Coil7_Position), extracted from a metadata file, outlining the antenna layout and maintenance logs (i.e. when antennas were changed). Coils of interest were merged accordingly, and the cleaned and processed data were exported to a CSV file for each pen. ‘Drift’ between the video cameras and the RFID technology was calculated manually each day when manual observations were paired with RFID data, i.e. NOT validation and piling observations. Drift was typically between 0 and 8 seconds.

At times, RFID readings indicated that hens were in different pens than originally placed. This occurred for one of two reasons: hens may have ‘jumped’ into the adjacent pen during caretaker health checks, or antennas in adjacent pens detected the hens close to the fence line. To account for these events, a

file outlining the location for each hen for each day of the production cycle was generated, defining the 'pen location' as the pen on that day where > 80% of her RFID detections were registered.

There were events where the RFID system malfunctioned, generally when there was significant interference between antennas, causing a malfunction in the data collection at individual coils (i.e. the precision required to capture a hen's involvement in a pile, or with an object (novel object or human) that reduced the reliability of the data. The data associated with these events were removed from the analysis. Six piles (all from one specific pen) were removed from the analysis due to this problem. Two further piles were removed from analysis due to impaired visual observations because the camera shifted.

2.4 Time spent on the litter

Time spent on the litter and the propensity to pile were investigated across all time points. The time spent on the litter, on the three days when piling was observed, was calculated and presented as a fraction of the total time spent on the litter.

2.5 Assessing behavioural phenotype

Phenotype assessment includes their response to novelty. Behavioural responses were assessed two days after piling observations.

2.5.1 Novel object test

The novel object test (NOT) was intended to assess individual consistency in responsiveness to a repeated, standardised stimulus across life stages, rather than generalised fearfulness to arbitrary novel objects. Consequently, time point effects reflect age and cumulative experience under a repeated exposure paradigm.

One novel object was positioned in each pen at each time point, placed in the aviary after the lights were turned off. Plastic panels (50 cm × 55 cm) were mounted on the pen walls to obscure visual and tactile access of adjacent pens to the NO. The panels were installed 7 days before the first time point of piling observations and were left for the duration of the experiment. A different NO was presented at each time point (Table 4).

The NOT objects were chosen to include novel and neutral objects to assess fearfulness (coloured stick and traffic cone) and novel stimuli that have been previously investigated in the context of piling and social attraction (light spots and mirror) (Table 4). Stimulus presentation was standardised (same location and stimulus) across life stages to maximise comparability and reduce contextual variation in large group-housed pens.

Table 4 The novel objects that were provided in each pen at each time point

Time point	Novel Object (NO)	NO properties	Adapted from
1	Coloured stick	Stacked building blocks vertically mounted 15–20 cm from the ground.	De Haas et al. 2014; van der Eijk et al. 2020
2	Acrylic mirror	200 mm × 200 mm acrylic mirror; 50 mm wide blue, green and yellow border.	Hedlund et al. 2021
3	LED lights	Strip of LED lights, 100 cm L; 5 cm wide with 60 LED bulbs. Four phases: off; stationary white light; white light moving in a pattern; coloured lights (green and yellow) in a random pattern. Each phase ran for 30 minutes. Lights moved at 12 cm/s.	Winter et al. 2022b
4	Traffic cone	Orange with two white stripes.	Larsen et al. 2018

The coils adjacent to the NO recorded the hens' approach over 6 days. The number of interactions with the NO on Day 1 was used as an index of initial responsiveness to the stimulus, and the number of total interactions over 6 days was included as further assessment of a hen's motivation to interact with each object. The time spent at the NO was calculated for each visit, and a visit was defined as any detection on the target coils. A visit was over when a hen was not detected on the target coil for at least 30 seconds.

2.6 Statistical analysis

Statistical analyses were completed with R Studio or Python. Two independent approaches were used to understand the piling behaviour and the relationships between piling behaviour, genotype and phenotype.

2.6.1 Flock piling characteristics over time

Comparisons between time points in flock piling characteristics were analysed with generalised linear mixed models (GLMM). The duration of piling events at each time point and the average hen entry and exit fractions (as a proportion of piling duration) were analysed with a gamma distribution. The number of piling events was analysed with a GLMM, with a Poisson distribution. The number of hens that participated in a pile was analysed with a Negative binomial GLMM due to overdispersion issues. All models included time point as a fixed factor and pen as a random factor.

2.6.2 Repeatability of piling behaviour

Piling behaviour was initially analysed as the absence or presence of each pile. Repeatability was calculated with the rptR package in R Studio and included the timepoint (age) as a fixed factor, and the pen and individual as a random factor. Repeatability analysis can determine the factors contributing to the variance in piling behaviour, specifically partitioning the inter- and intra-group sources of variance (i.e. between pens or time point (age), or within individuals), labelled as the intra-class correlation (ICC). Therefore, the repeatability of piling behaviour (R) was calculated by assessing the pen variance (V_G) over the sum of the pen variance and residual variance (V_R)

$$R = V_G / (V_G + V_R)$$

The statistical influence of factors on repeatability can be assessed by likelihood ratio tests, which compare the model fit when specific factors are excluded to determine the relative contribution of each factor of interest.

Individual participation in a pile, latency to join the pile and the time spent in the pile were analysed using mixed models, using a quadratic term of week of age to account for the non-linear relationship between age and piling as an explanatory variable, and individual identity, sire line and pen as random factors. Individual participation was fit with a binary distribution. For latency to pile, the total duration of the pile was added as an explanatory variable to control for the effect of duration of the pile on the latency to join the pile. The model was fit with a negative binomial distribution and included a zero-inflation parameter to account for the generation of zeroes that happened due to assigning zeros to hens detected 30 seconds before CR1. We used proportion of time spent in a pile, dividing it by the total duration of the pile, to account for the difference in length of piles, and used a Gaussian distribution to fit the model. To test the significance of the explanatory and random variables, we removed the variable of interest and compared it to the global model using likelihood ratio tests. We used lme4 and glmmTMB packages from R to perform the mixed model analysis.

All hens that died at any time during the production cycle were removed from this analysis.

To further investigate and quantify the consistency of individual hen's participation in piling events across observations, taking into account the location in the pen before the piling event, the repeatability (intra-class correlation (ICC) was estimated) by fitting a GLMM with a binary outcome (participated in a pile or not) and calculating repeatability on both the original and latent (link) scales, using the rptBinary() function from the rptR package in R. A series of models were run, including a random intercept for individual hen identity (RFID) to assess between-individual variance in participation (i.e. the null model), a model including the hen's location immediately before a pile (on the litter or not), and a final model that included location and time point interaction. Each model was bootstrapped with 1000 iterations to obtain 95% confidence intervals. No permutations were used. Likelihood ratio tests confirmed that the inclusion of the random effect significantly improved model fit in all cases ($p < 0.001$).

2.6.3 Bayesian inference

A Bayesian hierarchical model was used to estimate the effects of individual behavioural traits, sire (genetic background), and their interaction on the probability of an individual participating in piling events. Multiple model configurations were explored. The configuration with the best performance, measured by the Pareto smoothed importance sampling leave-one-out cross-validation (LOO) criterion was as follows:

$$\text{logit}(P_{ij}) = \alpha + \beta_j + \gamma(\tau_i)$$

Where:

- P_{ij} is the probability that an individual i , with sire j , is involved in a potential piling event.
- α represents a population-level baseline probability contribution.
- β_j is the probability contribution of sire j .
- γ models the contribution for an individual with a total novel object interaction duration of τ_i using a piecewise linear function with six cut points evenly spaced at the quintiles of the data.

The prior for α was a standard Normal distribution (zero mean and unit variance). β_j was drawn from a normal distribution with zero mean and a standard deviation of σ_β , a population-level hyper-prior, itself drawn from a standard Normal distribution. We modelled the values of the piecewise linear function γ at the six cut points, also using standard Normal priors. The final likelihood was assumed to follow a Beta-Binomial distribution, motivated by an observed over-dispersion of pile counts relative to a more commonly employed Binomial (see Figure 7):

$$L_i(N_i | n_i, P_{ij}) = \text{BetaBinomial}(n_i, \alpha_{bb} P_{ij}, (1 - P_{ij}) \beta_{bb})$$

Where:

- N_i is the number of piles in which an individual i participated.
- n_i is the total number of piles it could have participated in (given, for example, its pen location).
- α_{bb} and β_{bb} are overdispersion parameters drawn from a Gamma(1, 1) distribution.

Models were constructed and fit using the Python package PyMC, with its default No-U-Turn (NUTS) sampler. Four chains were run with 1000 tuning iterations followed by 1000 posterior sample draws. Convergence was assessed visually and by ensuring all chains possessed a Gelman-Rubin (R) value ≤ 1.01 .

3 Results

3.1 Flock piling characteristics

Across all five pens, 89 piling events were recorded over the 12 observation days (3 days of observation at each of the 4 time points) (Figure 3).

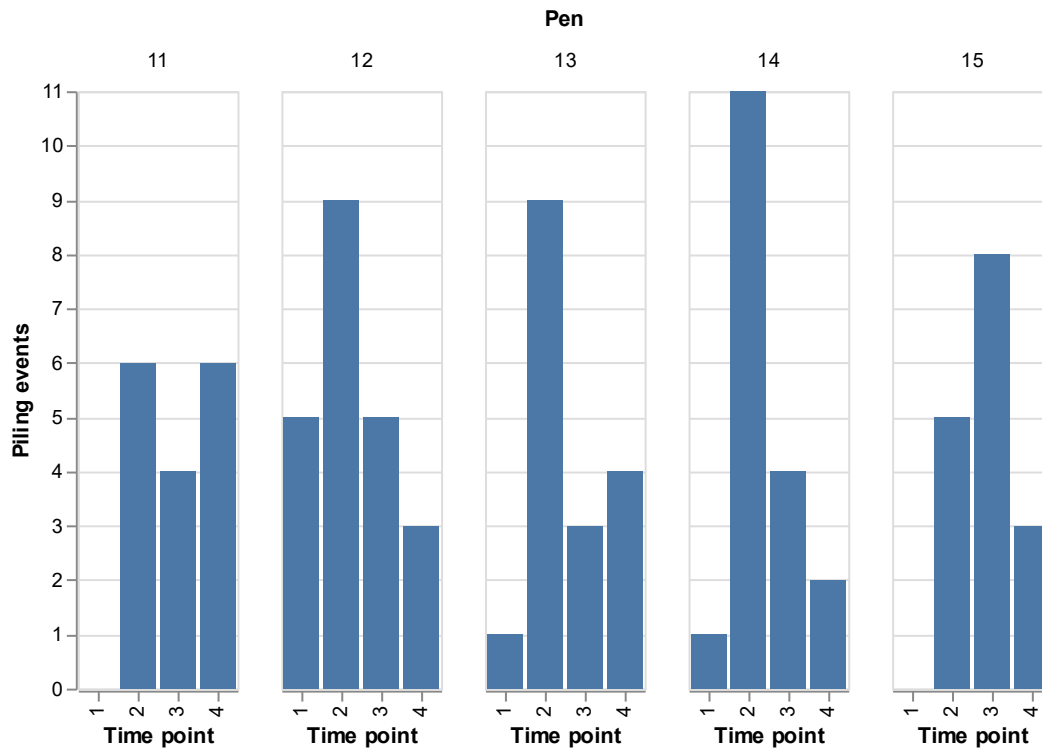


Figure 3 The number of piling events over three-day observation periods at four time points for each pen

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

The number of piling events peaked at time point two (Figure 3 and Figure 4). There were more piling events at time point 2 than at time point 1 ($\beta = -1.23$, $SE = 0.41$, $z = -3.01$, $p = 0.014$) and time point 4 ($\beta = 0.799$, $SE = 0.28$, $z = -2.81$, $p = 0.014$; Figure 4). There was no difference in the number of piling events between time points 1, 3 and 4 ($p > 0.05$; Figure 4).

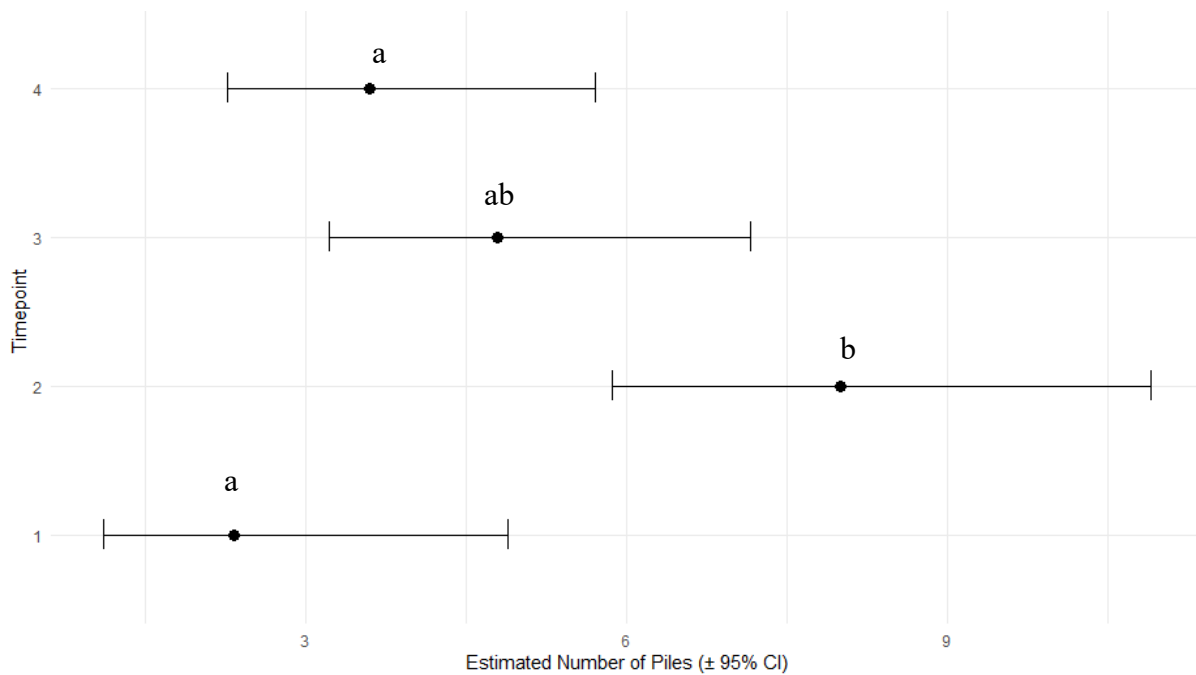


Figure 4 The estimated number of piling events at each time point based on a Poisson mixed model controlling for pen as a random factor

Points represent model-estimated means ($\pm$ 95% confidence intervals), averages across pens.

Differing subscripts indicate significantly different number of piling events between time points ($p < 0.05$).

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

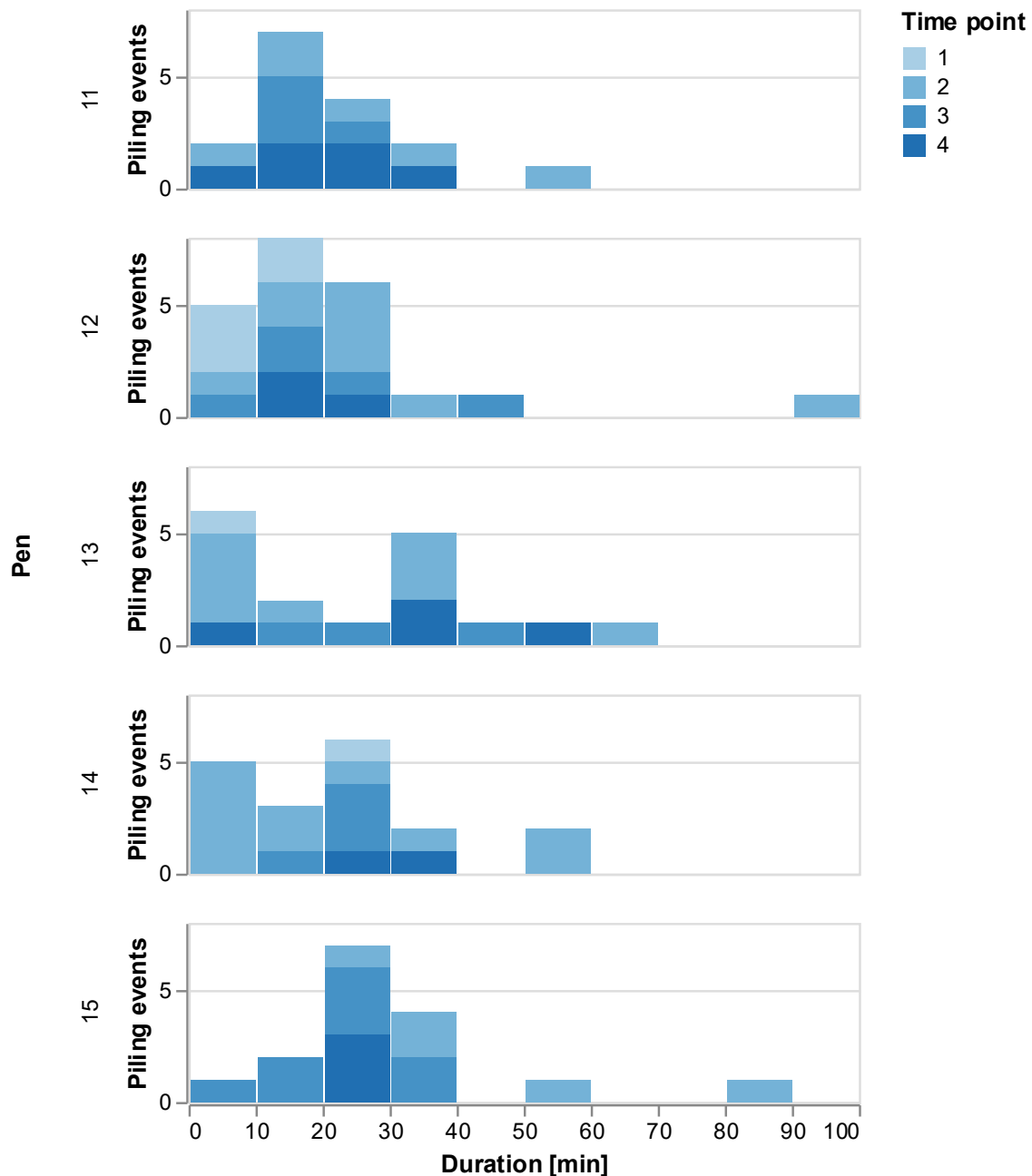


Figure 5 The duration of piles for each pen at 20, 30, 52 and 72 weeks of age (time points 1 – 4).

Shades of blue (light to dark) denote piling observed at each time point 1 to 4.

There was an age/experience effect on the duration of piling events ($\beta = 2.20$, $SE = 0.26$, $z = 8.47$, $p < 0.0001$). Piling events were shorter at time point 1 compared to all other time points (time point 2: $\beta = -1.07$, $SE = 0.28$, $z = -3.79$, $p < 0.001$; time point 3: $\beta = -0.87$, $SE = 0.30$, $z = -2.94$, $p = 0.017$; time point 4: $\beta = -1.00$, $SE = 0.31$, $z = -3.27$, $p = 0.005$; Figure 6). There was no difference in the duration of piles between time points 2, 3 and 4 (Figure 6).

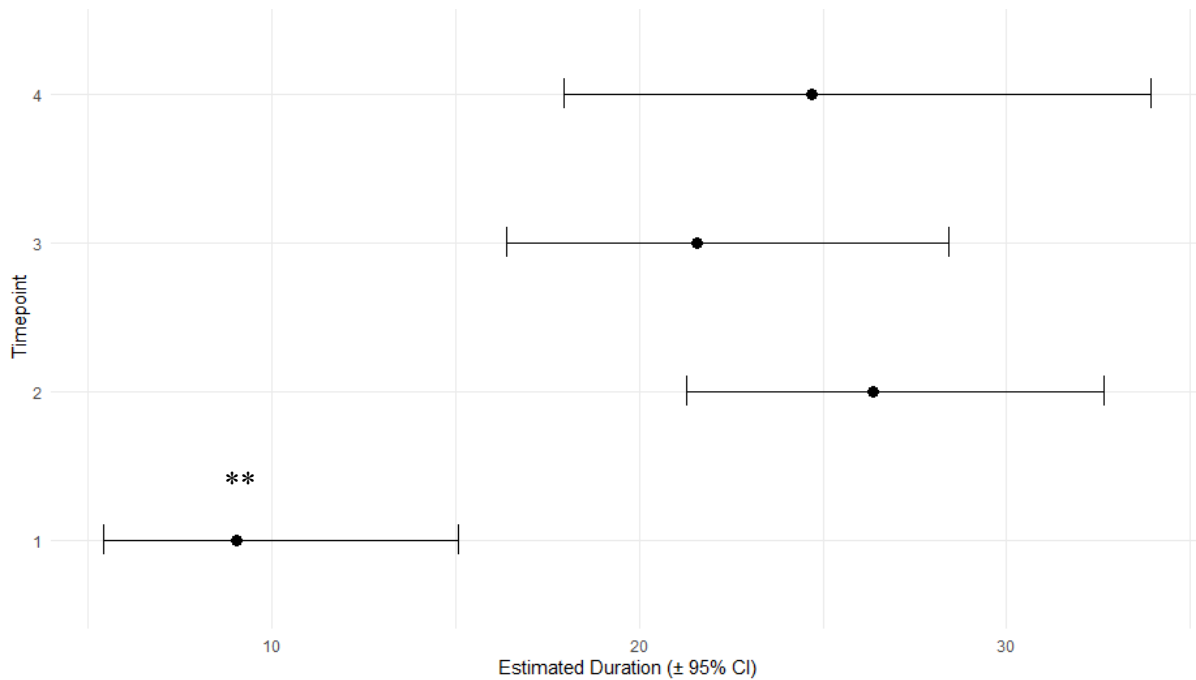


Figure 6 The estimated duration of a piling event at each time point from a GLMM gamma distribution, controlling for pen as a random factor

Points represent model-estimated means ($\pm$ 95% confidence intervals), averages across pens.

** indicates a significant difference in the duration of piling events between time points ($p < 0.01$).

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

The maximum number of hens in a pile at one time ranged between 4 and 85 hens. However, the maximum number of hens that participated in a pile (i.e. not necessarily at one time) ranged between 5 and 150 hens. The number of hens that participated in a pile differed across time points ($\beta = 2.87$, $SE = 0.23$, $z = 12.12$, $p < 0.0001$). There were significantly fewer hens participating in piling events at time point 1 than all other time points (time point 2: $\beta = -1.30$, $SE = 0.26$, $z = -5.08$, $p < 0.0001$; time point 3: $\beta = -1.61$, $SE = 0.27$, $z = -6.05$, $p < 0.0001$; time point 4: $\beta = -1.53$, $SE = 0.28$, $z = -5.57$, $p < 0.0001$; Figure 7). There was no difference in the number of hens that participated in a piling event at time points 2, 3 or 4 (Figure 7).

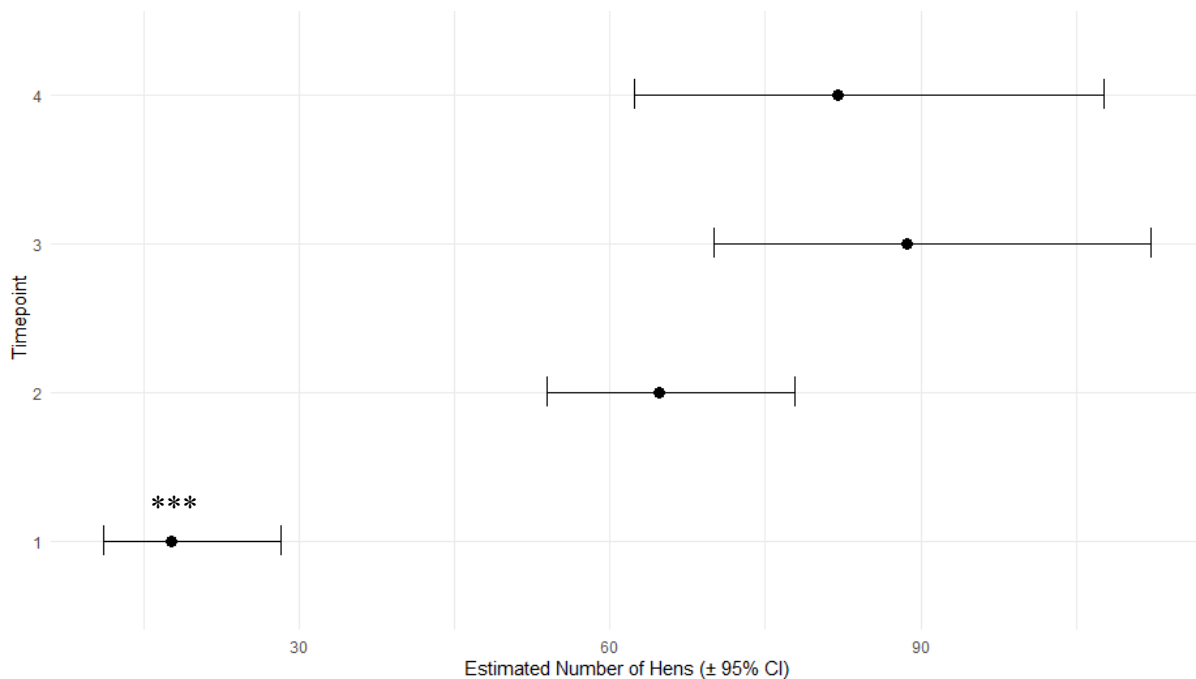


Figure 7 The estimated number of hens that participated in a piling event at each time point from a GLMM controlling for pen as a random factor

Points represent model-estimated means ($\pm$ 95% confidence intervals), averaged across pens.

*** indicates a significant difference in the duration of piling events between time points ($p < 0.001$).

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

The latency for a hen to join a piling event was normalised to account for the different duration of piling events and is expressed here as the entry fraction. Entry time was determined as the elapsed time between pile initiation (defined in table 2 and 3) and the first RFID detection of an individual hen within the identified pile area. There was a significant effect of time point on the average entry fraction ($\beta = 0.28$, $SE = 0.03$, $z = 9.28$, $p < 0.0001$). The average entry fraction ranged between 0.20 and 0.28, with more hens taking longer to join a pile at time point 2 than at time point 3 ($\beta = 0.06$, $SE = 0.02$, $z = 2.6$, $p = 0.048$; Figure 8).

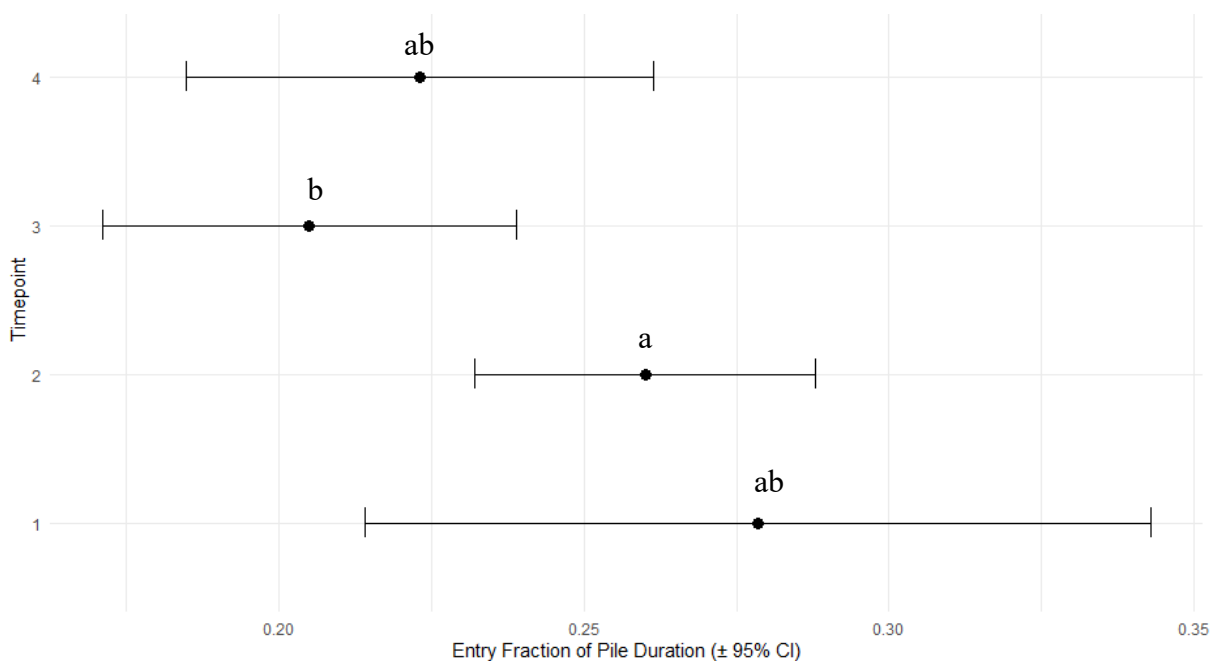


Figure 8 The estimated average entry fraction for hens that participated in a piling event at each time point from a GLMM controlling for pen as a random factor

Entry fraction was calculated as the elapsed time between pile initiation and the first RFID detection of a hen within the pile area, expressed as a proportion of the total pile duration. Points represent model-estimated means ($\pm$ 95% confidence intervals), averaged across pens.

Differing substrate indicates a significant difference between time points ($p = 0.05$).

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

The time a hen left a pile was normalised to account for the different duration of piling events and is expressed here as the exit fraction. An individual hens exit time was recorded as the last RFID detection in the piling area before the piling event stopped, i.e., the criteria outlined in table 2 and 3 were no longer met. The average exit fraction differed across time points ($\beta = 2.18$, $SE = 0.25$, $z = 8.82$, $p < 0.001$). The average exit fraction was calculated for each hen by dividing the time that a hen left a pile as a fraction of the overall duration of the piling event. Hens took longer to exit a pile at time point 1 than at time point 2 ($\beta = 0.97$, $SE = 0.26$, $z = 3.75$, $p = 0.001$), 3 ($\beta = 1.15$, $SE = 0.26$, $z = 4.34$, $p = 0.0001$) and 4 ($\beta = 1.04$, $SE = 0.27$, $z = 3.84$, $p < 0.001$).

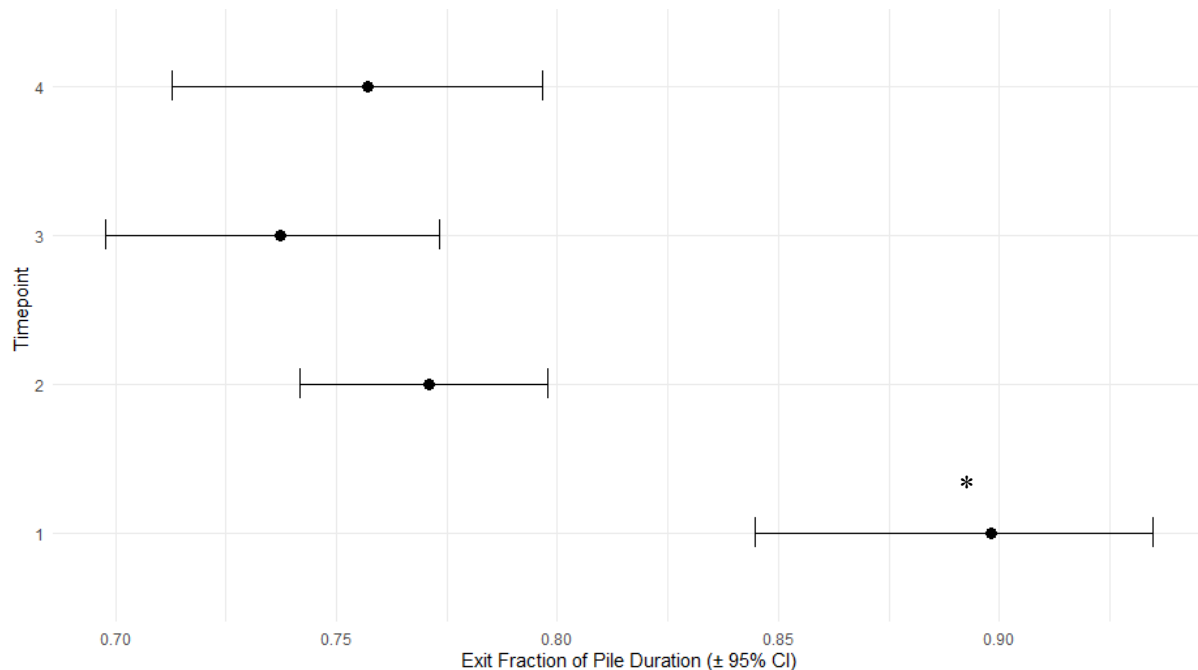


Figure 9 The estimated average exit duration fraction for hens that participated in a piling event at each time point from a GLMM with Beta distribution, controlling for pen as a random factor

The exit fraction was calculated as the elapsed time between the last RFID detection within the pile area and the end of the piling event, and expressed as a proportion of the total pile duration

Points represent model-estimated means ($\pm$ 95% confidence intervals), averaged across pens.

* indicates a significant difference in the duration of piling events between time points ($p = 0.05$).

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

3.2 Individual piling behaviour

Less than 15% of hens participated in a piling event during the first observation period. This increased to more than 25% in the second observation period, and slightly below 50% of hens participated in a piling event in the third and fourth observation periods (Figure 10).

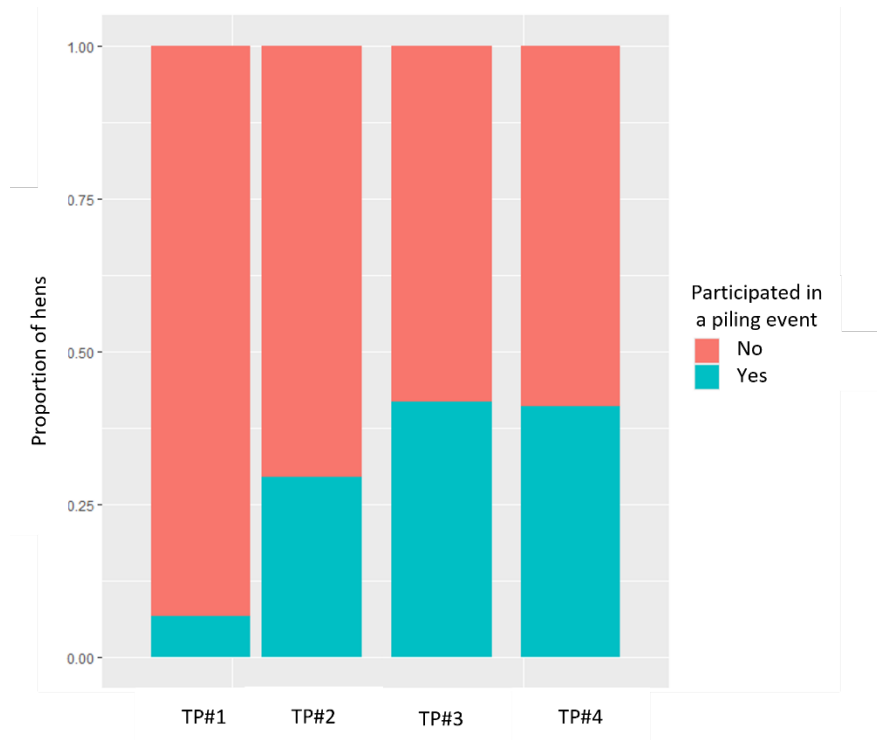


Figure 10 The proportion of hens that participated in a piling event across four time points at 20 (TP#1), 30 (TP#2), 52 (TP#3), and 72 (TP#4) weeks of age

Throughout the four observation periods across the production period, 120 hens (~10% of hens) did not participate in any of the observed piling events (Figure 11). The remaining hens' pile participation ranged from 1 to 15 piles (out of a maximum of 17–22 piling events). Most hens participated in 1 to 9 piling events (Figure 11).

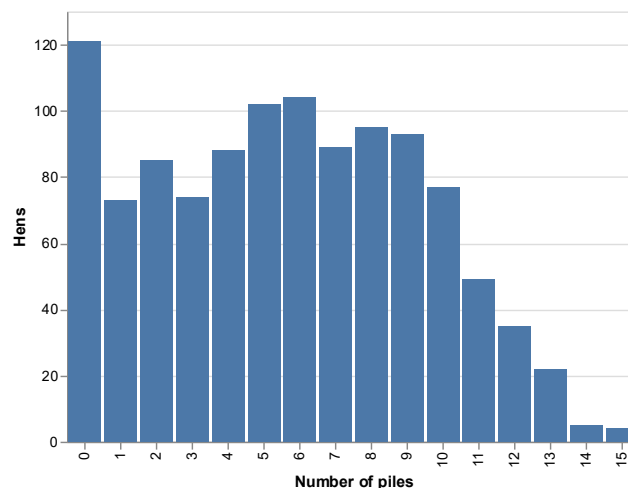


Figure 11 The distribution of the number of piles that individual hens participated in

The probability density of time spent in a piling event (i.e. how likely an individual would spend in a pile) showed that in most piles, at least one hen participated for a very short time. However, most hens spend between 5 and 15 minutes in a pile. The maximum duration curve suggests that in many piling events, some hens spent a long time in the pile, with a few rare cases showing that an individual hen spent up to 90 minutes in a pile (Figure 12).

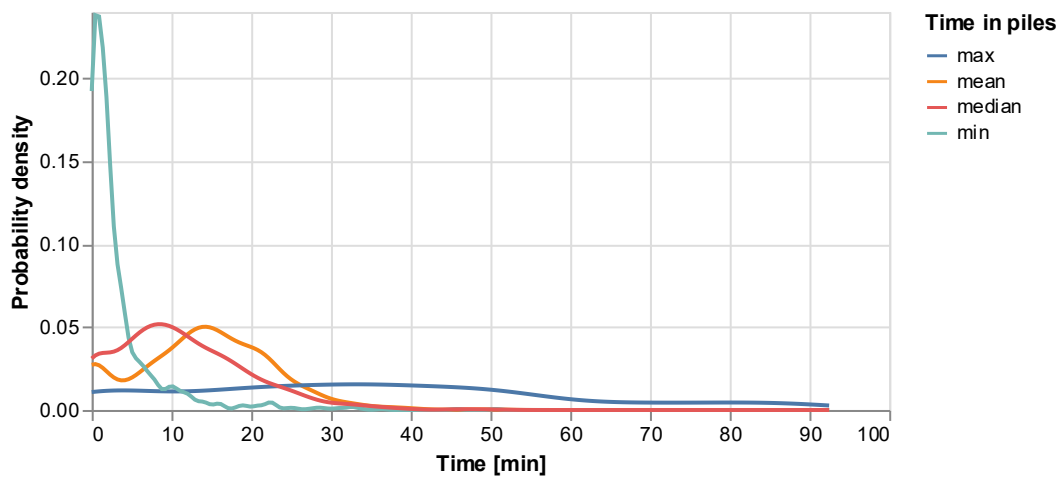


Figure 12 A density plot showing the distribution of the minimum (blue), maximum (dark blue), mean (orange) and median (red) time a hen spent in a piling event

The y-axis reflects the relative likelihood of the duration spent in a pile.

Hens typically exited a pile in a similar order to that in which they joined, with many hens entering or leaving early in a piling event (Figure 13). Although a positive relationship existed between the entry and exit rank, the spread of the data indicates individual variation; some hens stayed longer than expected, while others exited the pile earlier than expected (Figure 13). These deviations suggest that certain hens may be especially motivated to pile, or conversely, reluctant to remain (Figure 13).

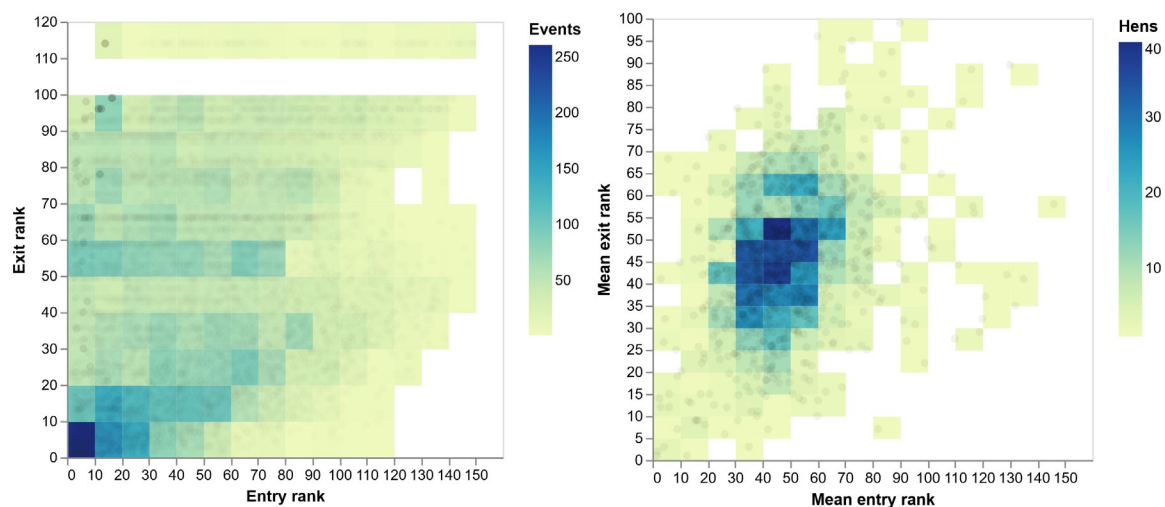


Figure 13 Heat maps showing the order (rank) in which a hen entered and exited a piling event for each event (left) and the average rank for each hen across all piles she participated in (right)

The darker colour squares indicate more data points.

Most hens entered a piling event early, within the first 5% of the pile duration, and exited late, within the final 5% (Figure 14). Early exits were rare, suggesting that regardless of when a hen joins, she tended to remain until the pile dissolved. Late recruitment was also uncommon, suggesting that piling is primarily established early in its duration. A smaller group of hens entered late and exited soon after (upper-right region of Figure 14), which may represent a hen's aversion to the pile conditions.

Individual average entry and exit time as a function of pile duration showed a repeatable, early-and-long participation behaviour across most hens.

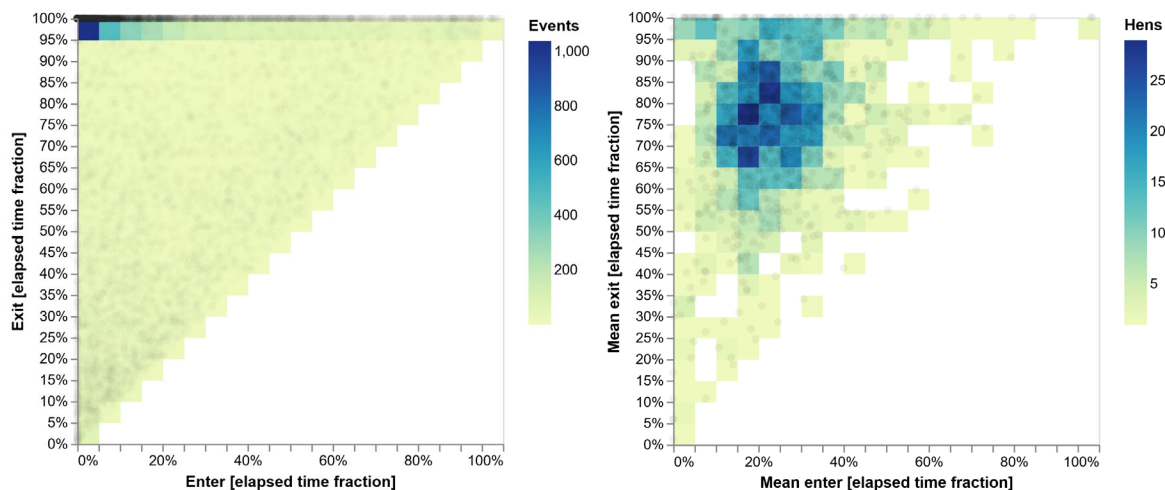


Figure 14 The fraction of total pile duration at which a hen entered (0% start of pile; 100% end of pile) and the fraction of total pile duration at which a hen exited

The left panel is per event; the right panel shows the mean values for each hen. The darker colour squares indicate more data points.

3.2.1 Location immediately before a piling event

Hens may have participated in a piling event if they had been located on the litter immediately before the pile started. Between 52 and 60% of hens that participated in a pile were on the litter immediately before a pile started (Figure 15).

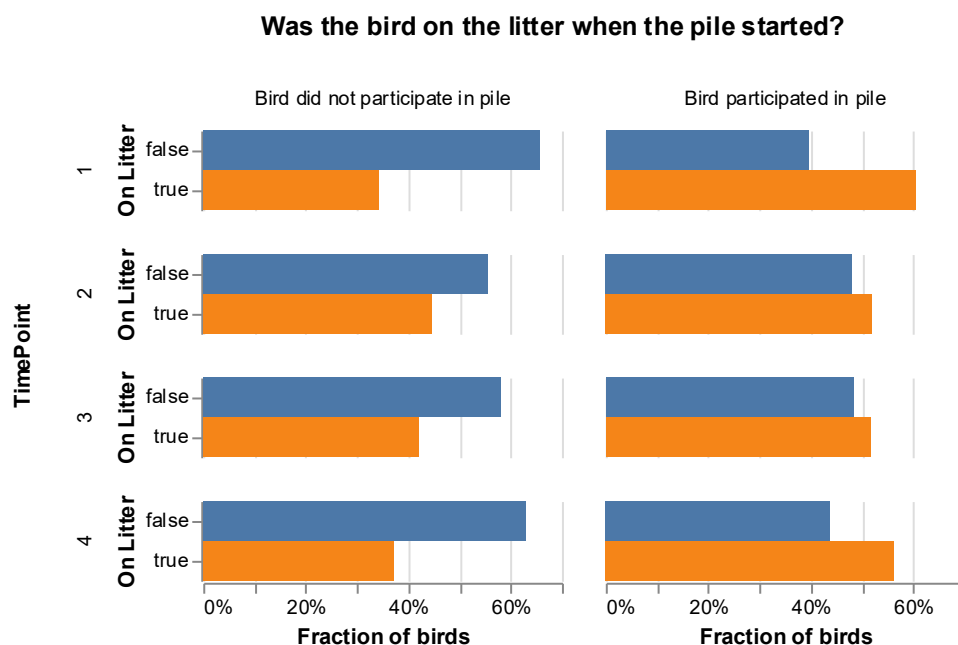


Figure 15 The proportion of hens that participated in a pile (right) or not (left) that were located on the litter (orange bars) or not (blue bars) immediately before the pile started

Hens with a low piling probability across all periods were found on the litter < 30% of the time before a piling event (Figure 16).

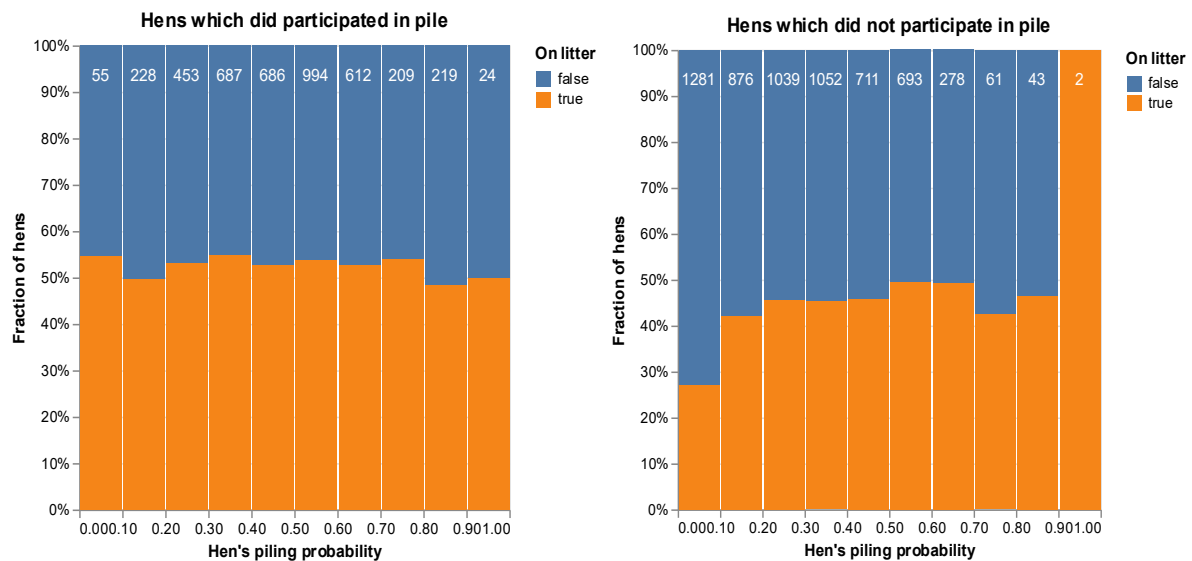


Figure 16 The proportion of hens that were found on the litter immediately preceding a piling event (orange bars) or not (blue bars), and the propensity to join a pile (left) or not (right)

The number of hens in each piling probability category is noted in white writing at the top of each bar.

Note the small sample size in the highest and lowest probabilities (apart from in the lowest for hens not participating).

Hens located on the litter immediately before a pile began were more likely to join the pile (β 1.29; SE 0.28, z 4.6, $p < 0.0001$). On average, hens on the litter were over three times more likely to participate in a piling event compared to hens that were not on the litter (OR = 3.64). As hens aged, the likelihood of joining a pile significantly increased, even if they were not on the litter. However, the effect of being on the litter was less pronounced over time (Figure 17).

Among hens that were not on the litter before the piling event, the odds of joining a pile at time point 1 were lower compared to time point 2 (OR 0.11, z -4.67, $p < 0.001$), time point 3 (OR 0.05, z -5.95, $p < 0.0001$) and time point 4 (OR 0.06, z -5.61, $p < 0.001$). Participation at time point 2 was also lower than time point 3 (OR 0.48, z -2.63, $p = 0.04$) but not time point 4 ($p = 0.140$). There was no difference in the likelihood of participation between time points 3 and 4 ($p = 0.996$).

For hens already on the litter, the odds of joining a pile at time point 1 were lower than time point 2 (OR 0.31, z -2.57, $p = 0.05$), time point 3 (OR 0.12, z -4.44, $p < 0.0001$) and time point 4 (OR 0.09, z -4.75, $p < 0.001$). Participation also increased from time point 2 to time point 3 (OR 0.39, z -3.39, $p = 0.01$) and time point 4 (OR 0.31, z -3.89, $p < 0.001$). There was no difference in participation between time points 3 and 4 ($p = 0.880$; Figure 17).

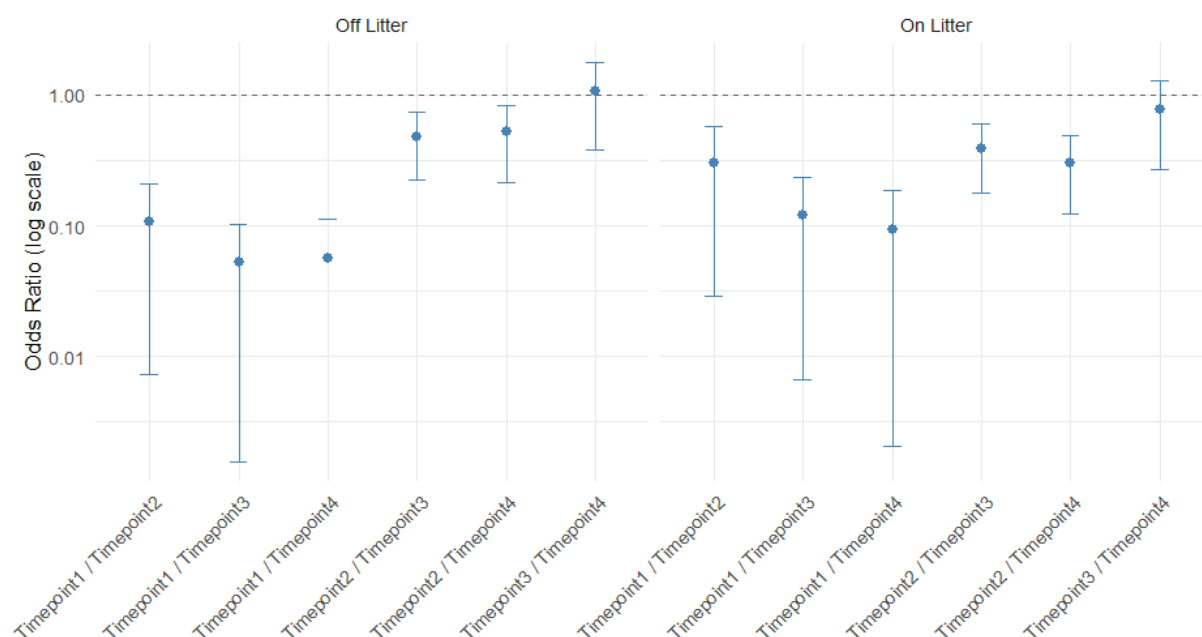
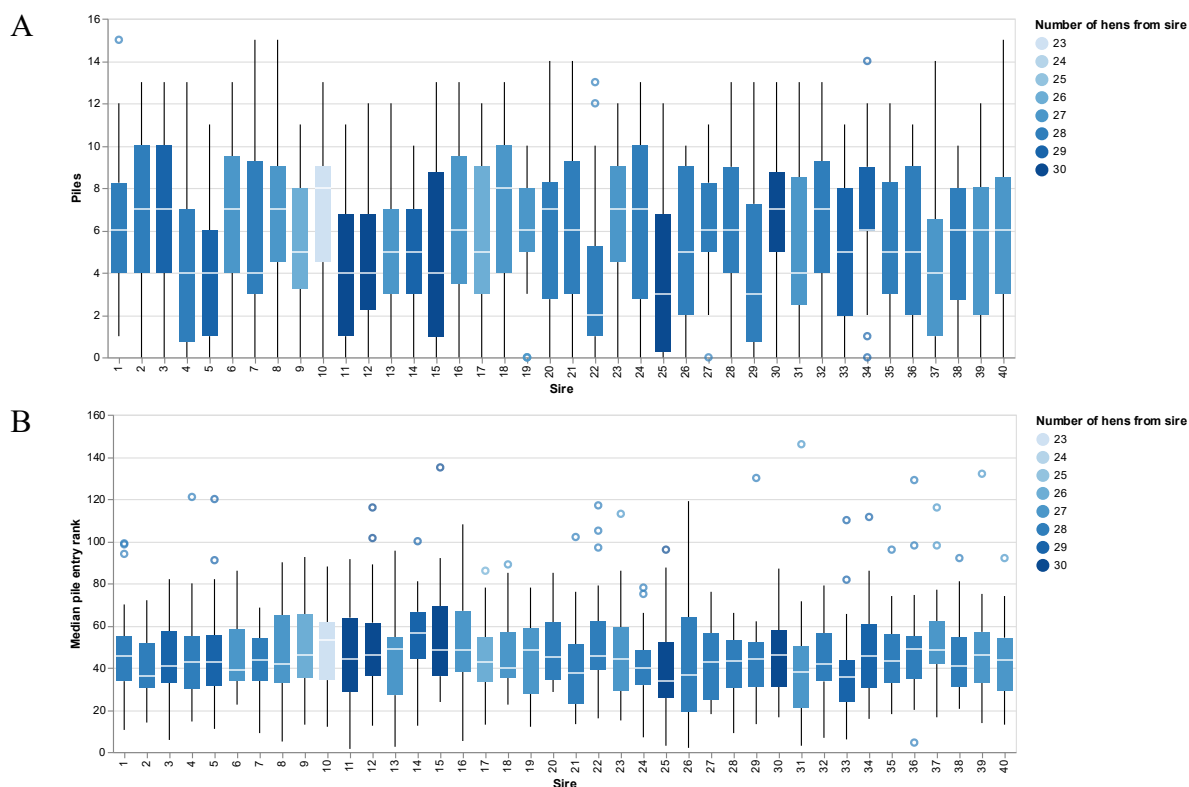


Figure 17 Comparisons of the odds ratio of a hen participating in a pile, if she was not on the litter (left) or was on the litter (right) immediately before a piling event at different time points

The dotted line represents equal odds of a hen participating in a pile between the two time points (odds ratio = 1.0). Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

3.2.2 Genetic relationships with piling

There was variation in piling behaviour between hens from different sires (Figure 18).



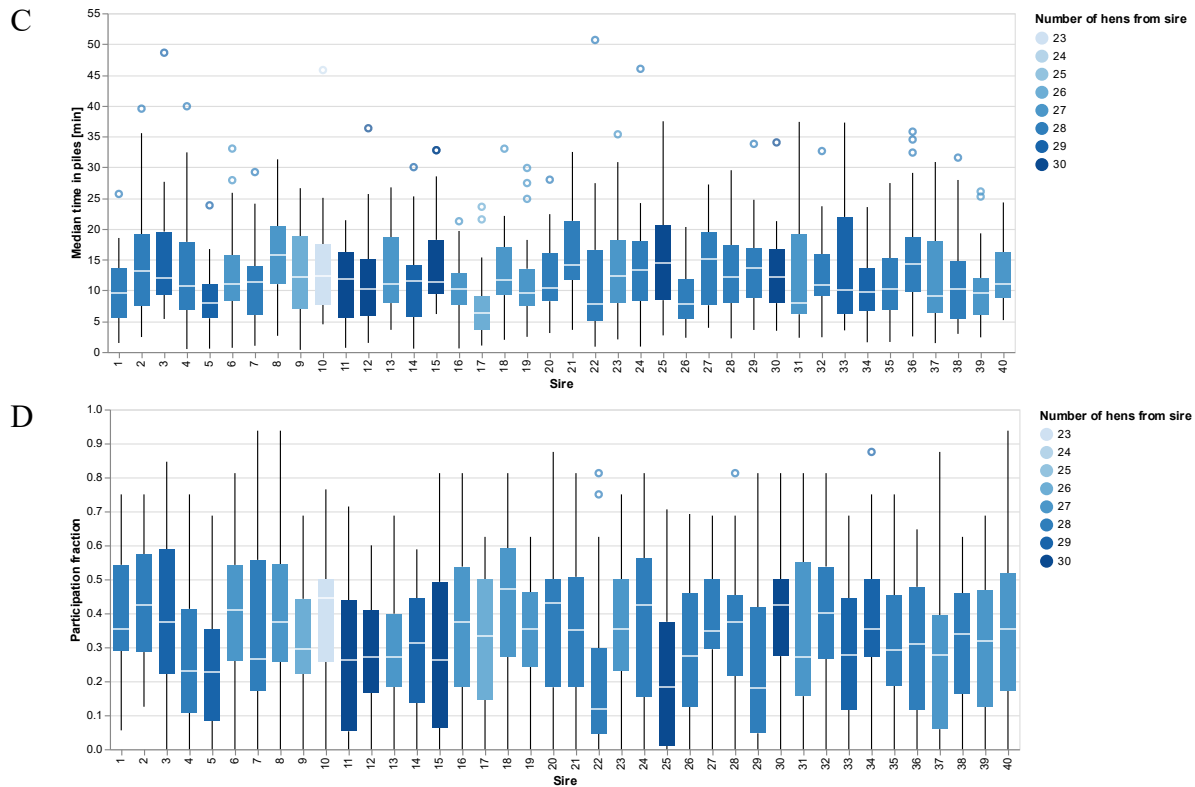


Figure 18 The number of piles (A), time spent in a pile (B), order of joining piles (C), and the participation fraction (D) for hens observed across four observation timepoints in the production cycle, relative to their sire

The number of hens represented by sire ranged from 23 to 30 and were randomly allocated to groups across five pens. The Tukey boxes indicate the 25th–75th interquartile range. White lines within the boxes indicate the median. Whiskers extend past the boxes by 1.5 times the inter-quartile range. The circles show the remaining outliers. Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

3.2.3 Repeatability estimates of piling behaviour

Repeatability analysis showed that inter-individual variation accounted for around 15% of the variation in piling behaviour. Although the effect of sire and pen on repeatability of piling behaviour was statistically significant, they explained very little of the variance (sire: 1.2%, pen: 1.9%; Table 5).

Table 5 The impact of sire, pen and individual hen on the likelihood that a hen would repeatedly join a pile

Source of variation	N	Repeatability	SE	95% CI	Likelihood ratio test (χ^2 , df, p)
Individual	1,142	0.152	0.0099	0.127 – 0.165	1180, 3, < 0.0001
Pen	5	0.019	0.0112	0.0010 – 0.0432	83.8, 1, < 0.0001
Sire	40	0.012	0.0042	0.0044 – 0.0211	25.3, 1, < 0.0001

Sire or individual did not predict the latency to join a pile ($p > 0.05$), or the time spent in a pile ($p > 0.005$). However, time point (age) significantly predicted how long hens spent in a pile, showing that hens spent longer in a pile at successive time points throughout the study (age; Figure 13).

A linear mixed model showed very low repeatability in the time hens joined a pile (entry time as a fraction of pile duration; ICC = 0.007), and only slightly higher consistency in the order they exited (exit time as a fraction of pile duration ICC = 0.033). This suggests that the order in which individual hens joined or left a pile was not strongly consistent across events.

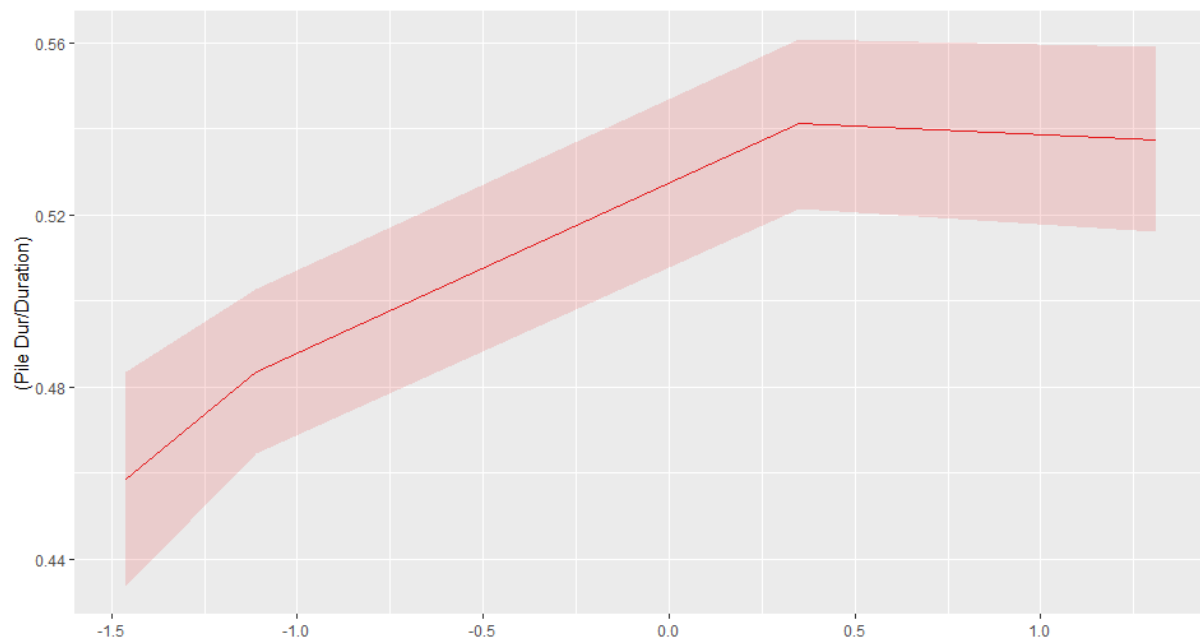


Figure 19 Predicted values for how long a hen spent in a pile as a function of observation timepoints/weeks of age (WoA)

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

Time point/age also influenced the latency to join a pile, with hens joining piles faster with increasing age ($\chi^2_{(2)} 17.33$, $p < 0.001$; Figure 20).

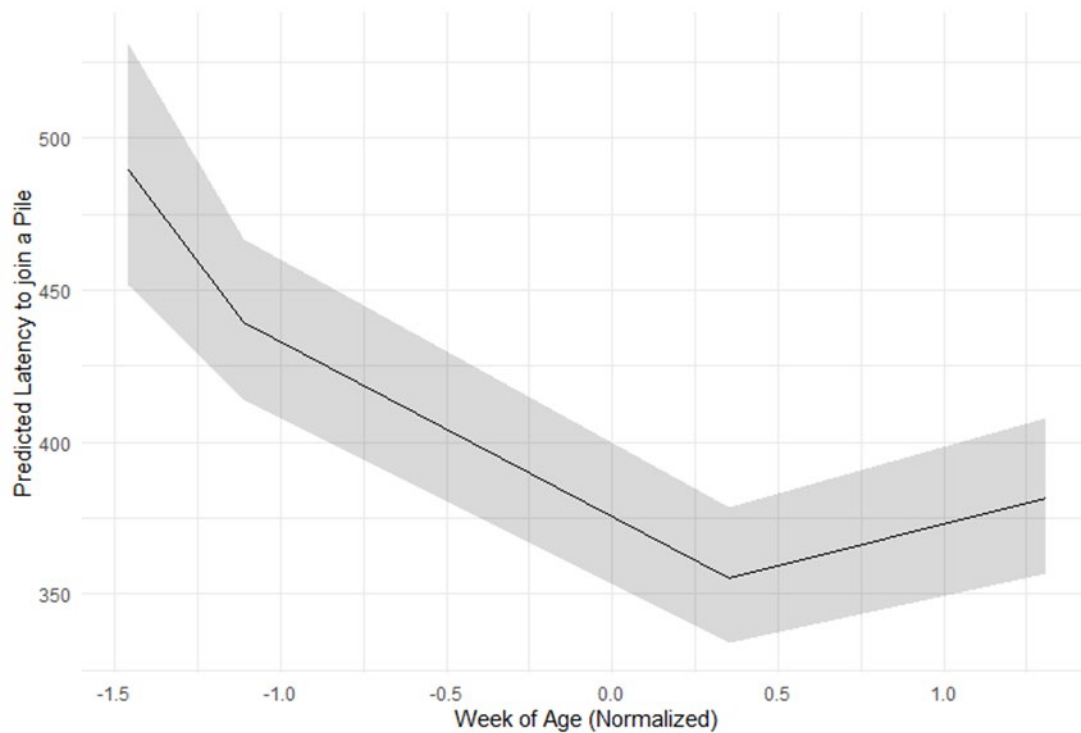


Figure 20 Predicted latency for hens to join piles with age

To further explore piling participation, we grouped hens into ‘early joiners’ if they were in the first 10% of hens to join each pile. Ten hens were identified as ‘early joiners’ in 100% of the piling events that they participated in (between 5 and 13 piles).

3.2.4 Repeatability of hens piling irrespective of location

There was moderate consistency in individual hens’ participation in piling events. In a null model, individual differences accounted for 19% of the variance in behaviour. Accounting for whether the pile occurred on litter, repeatability dropped to 14%, indicating that some individual variation is driven by environmental context. In the final model, time point and its interaction with location (i.e. on the litter) were also included and further reduced repeatability to 12% (Table 6).

Table 6 The impact of location before a piling event (on the litter or not), and interaction between time point and location before a piling event on the likelihood that an individual hen would repeatedly join a pile

Source of variation	Repeatability	SE	95% CI	Likelihood ratio test (χ^2 , df, p)
Individual	0.187	0.009	0.166 – 0.201	1600, 1, < 0.0001
Location before a piling event	0.143	0.010	0.117 – 0.154	468, 1, < 0.0001
Time point × location before a piling event	0.117	0.007	0.099 – 0.127	403, 1, < 0.0001

3.3 Novel object test

There was only one hen that never interacted with a NO at any time point. There were only 17 hens that never interacted with a NO on the first day at three out of the four time points. The number of interactions an individual hen had with the novel objects over 7 days at different time points (β 0.931, SE 0.02, $z = 158.9$, $p < 0.001$). Hens interacted more frequently with the NO at time point 4 and the least at time point 3 (Figure 21).

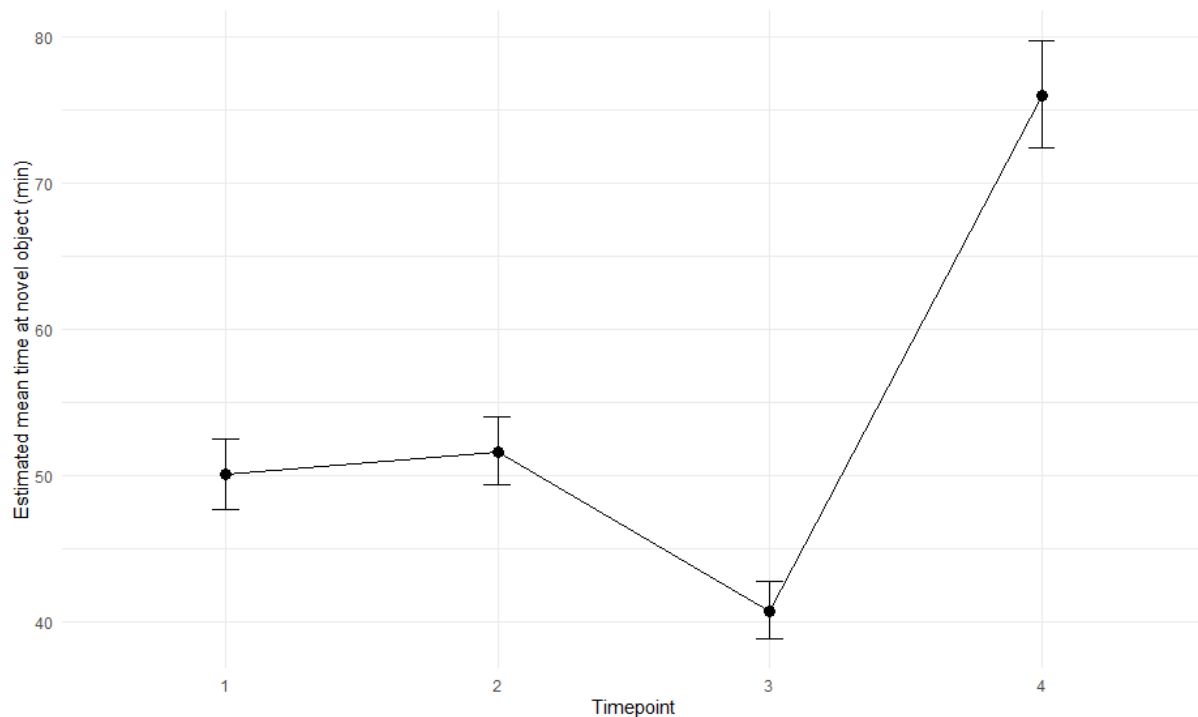


Figure 21 Estimated marginal means of time spent with the novel object by an individual hen at four observation time points

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

The latency to first interact with the novel object differed across time points, with longer latencies observed at time point 3 relative to all other time points. The latency to first approach was faster at time point 4 than time point 2 ($\beta = 1.20$, SE 0.07, $z = 3.25$, $p < 0.01$; Figure 22).

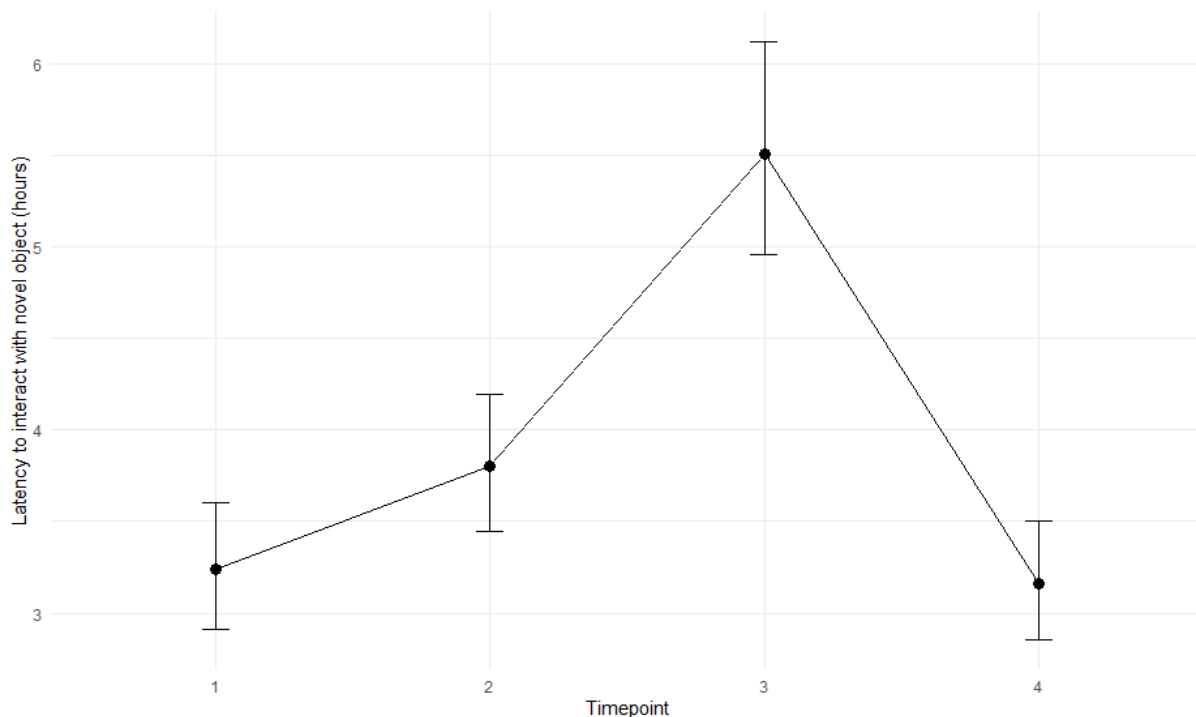


Figure 22 Estimated marginal means of time taken to first approach the novel object (latency) for an individual hen at four observation time points

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

There was variation in the total amount of time that individuals spent interacting with the novel objects when splitting the population by sire (genotype). However, there was a large variation within each sire group (Figure 23).

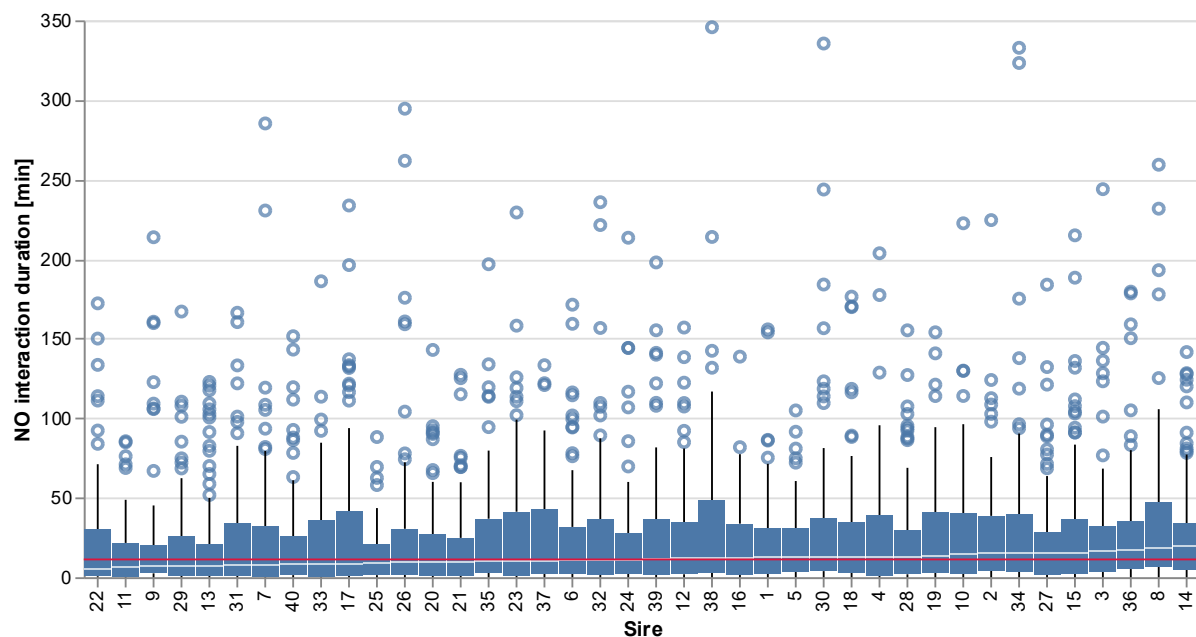


Figure 23 The total novel object (NO) interaction duration for each individual hen, split by sire

The Tukey boxes indicate the 25th–75th interquartile range.

White lines within the boxes indicate the median.

Whiskers extend past the boxes by 1.5 times the inter-quartile range.

The circles show the remaining outliers.
The red horizontal line shows the population median.

There were also differences between hens grouped by sire when data were further split into the novel object interaction duration by observation time points (Figure 24). In addition, there was variation in the typical amount of time spent interacting with novel objects at each time point (Figure 24). Most notably, individuals tended to spend more time interacting with the novel object at time point 2 (mirror) than at any other time point, indicating higher engagement with this stimulus under the conditions tested.

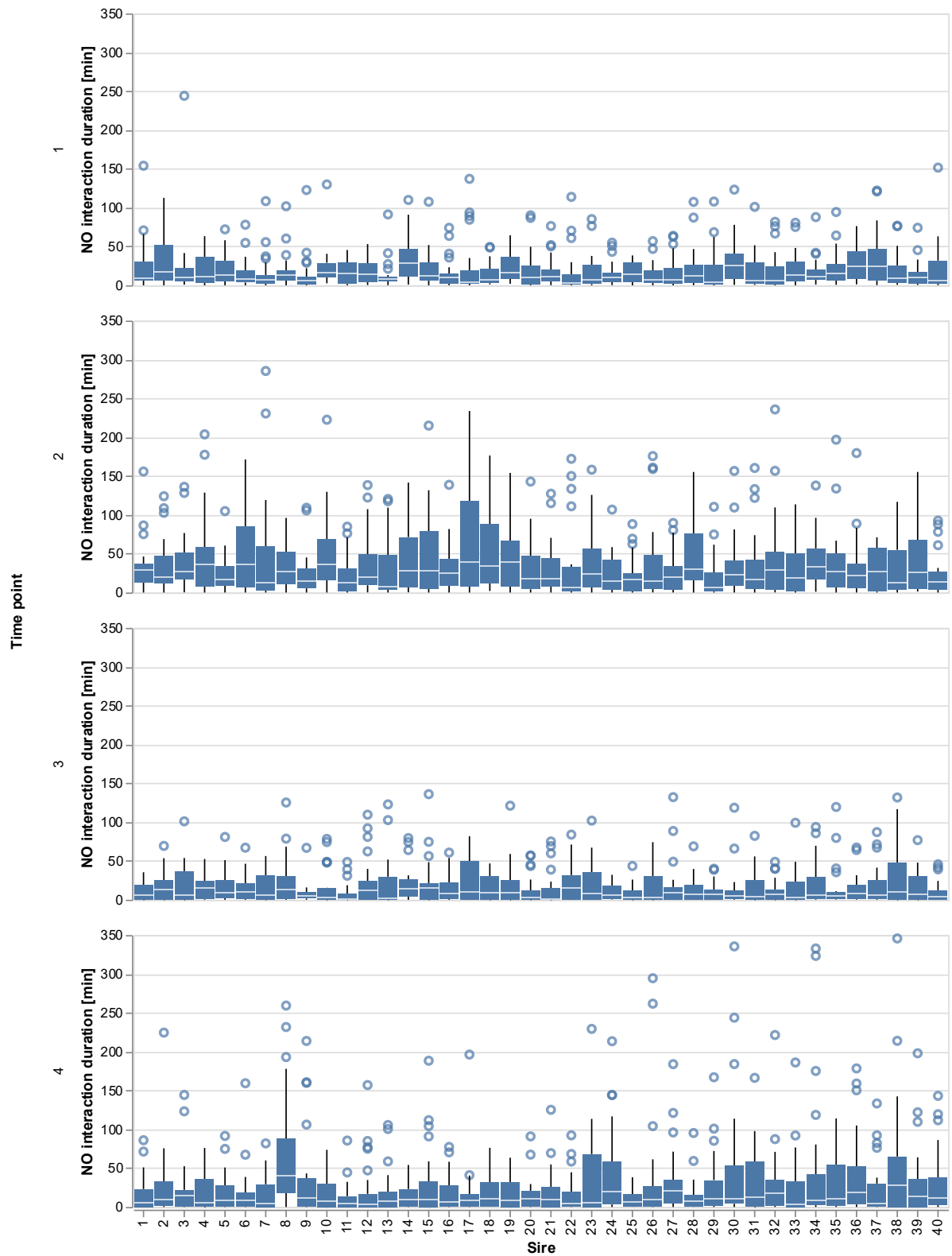


Figure 24 The total novel object (NO) interaction duration for individuals split by sire and time point

The Tukey boxes indicate the 25th–75th interquartile range.

White lines within the boxes indicate the median.

Whiskers extend past the boxes by 1.5 times the inter-quartile range.

The circles show the remaining outliers.

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

To remove noise arising from the difficulty in assigning membership to short-duration piles, we removed all piles that lasted less than 20 minutes for the remainder of the results presented in this section (50%; $n = 48$ piles removed). When determining the duration of novel object interactions, detections separated by more than 30 seconds were considered a new visit/event.

There was a clear trend in the total time spent interacting with the novel object when individuals were split by the total number of piles that they participated in during the observation days (Figure 25). Hens that interacted with the novel objects more typically participate in more piles.

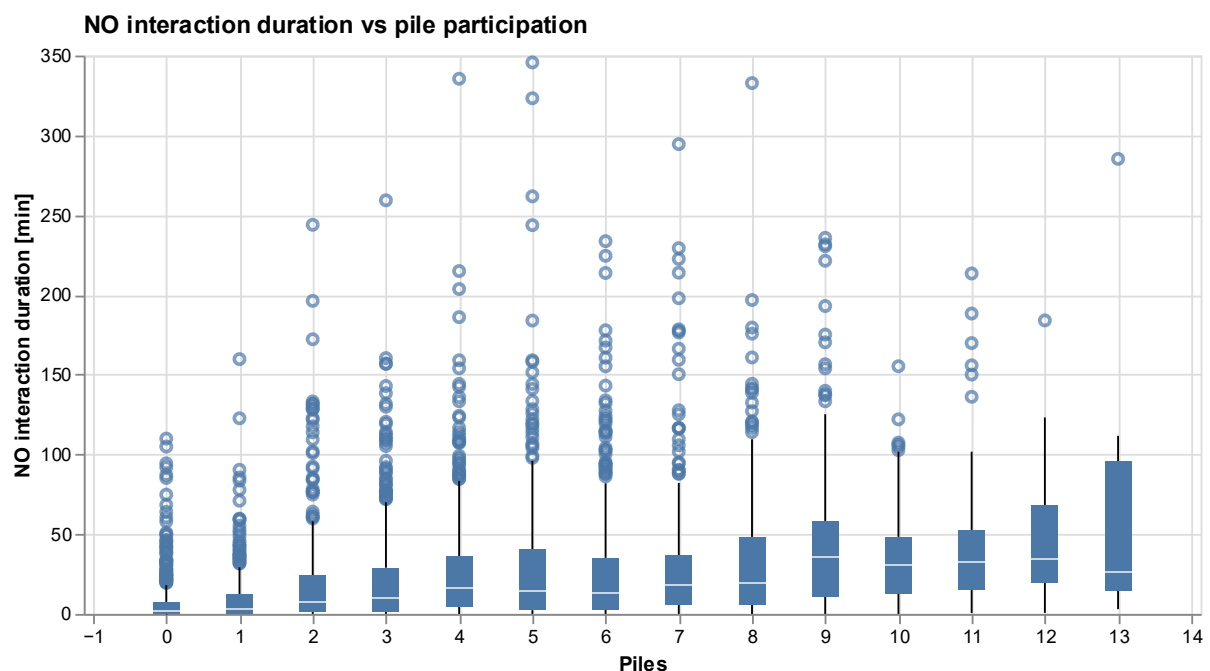


Figure 25 The total novel object interaction duration as a function of the number of piles (lasting longer than 20 minutes) each individual participated in

The interaction between novel object interactions and piling behaviour remains when the data were further split by time point. However, there was variation in the strength of the correlations. For example, there was no interaction between piling and NOT interactions at time point 1, whereas time point 2 showed the strongest correlation. Remembering that the time point 2 novel object was also the one most interacted with overall, this suggests that the importance of novel object interaction in determining piling behaviour is affected either by age and/or the type of novel object.

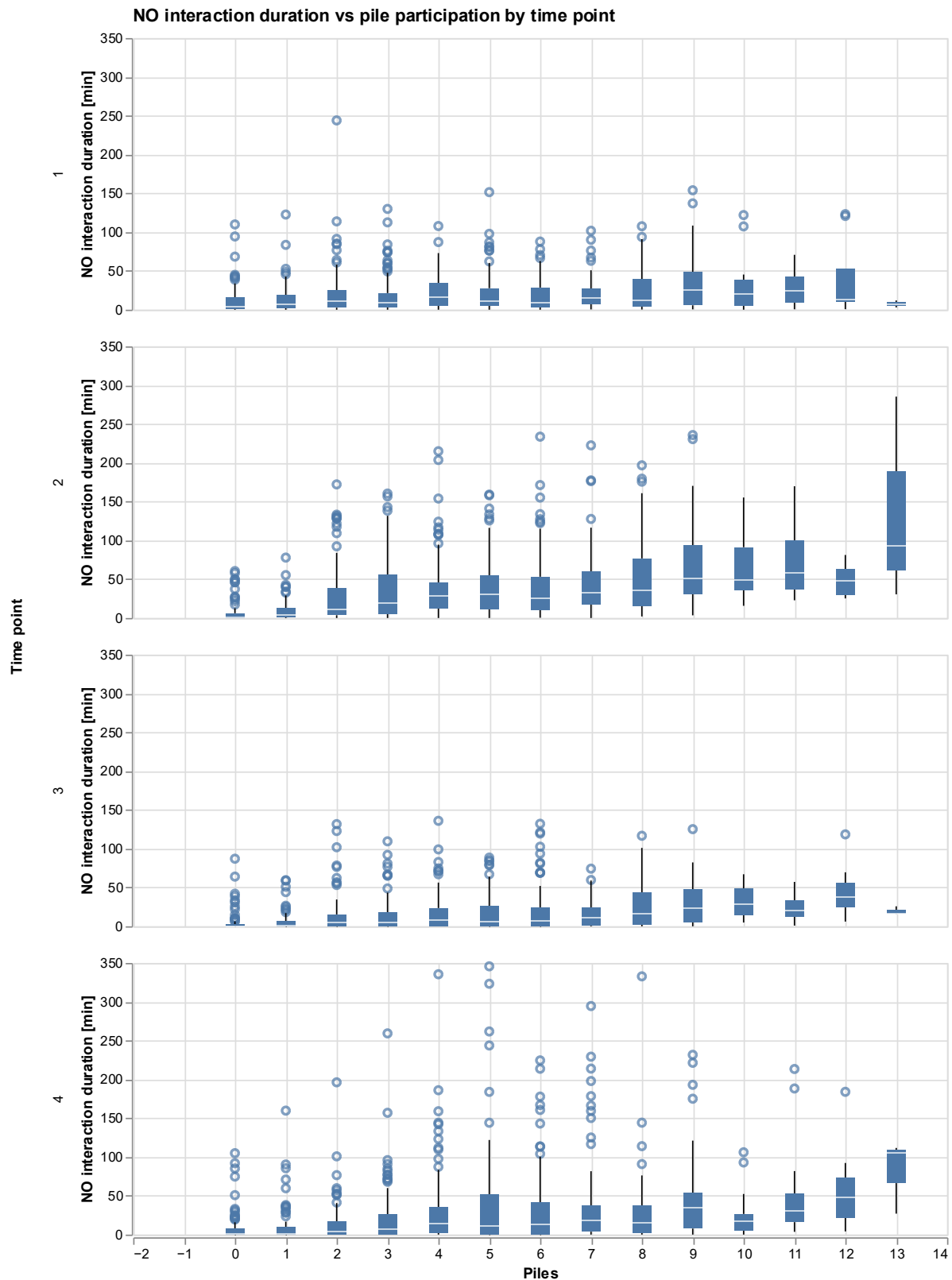


Figure 26 The total novel object interaction duration as a function of the number of piles (lasting longer than 20 minutes) each individual participated in, split by time point

Time points 1 – 4 correspond to behavioural observations conducted at 20, 30, 52 and 72 weeks of age.

3.3.1 Repeatability estimates of novel object interactions

The estimated repeatability of interaction with the novel object was small but significant ($R = 0.146$, $SE = 0.008$, 95% CI 0.131, 0.161, $p < 0.001$), indicating that 14.6% of the variation in time spent with the novel object was due to the individual. Therefore, environmental and temporal factors account for most of the variation; there is a stable individual component, suggesting that some hens are more curious/exploratory than others.

The estimated repeatability of an individual hen's latency to approach a novel object was significant but weak ($R = 0.069$, $SE = 0.019$, 95% CI 0.031, 0.104, $p < 0.001$), suggesting that less than 7% of the variance was explained by the individual hen.

3.4 The relative effects of genotype and behavioural phenotype propensity to pile

The absolute and relative importance of behavioural phenotype and genotype were assessed using our generative Bayesian model. Figure 27 shows the posterior predictive probability distribution for the number of piles an individual participates in, compared with the measured distribution from the data. This demonstrates that our model does realistically capture the true underlying distribution as well as a sensible level of scatter given the amount of variability in the underlying data (as demonstrated in the above plots).

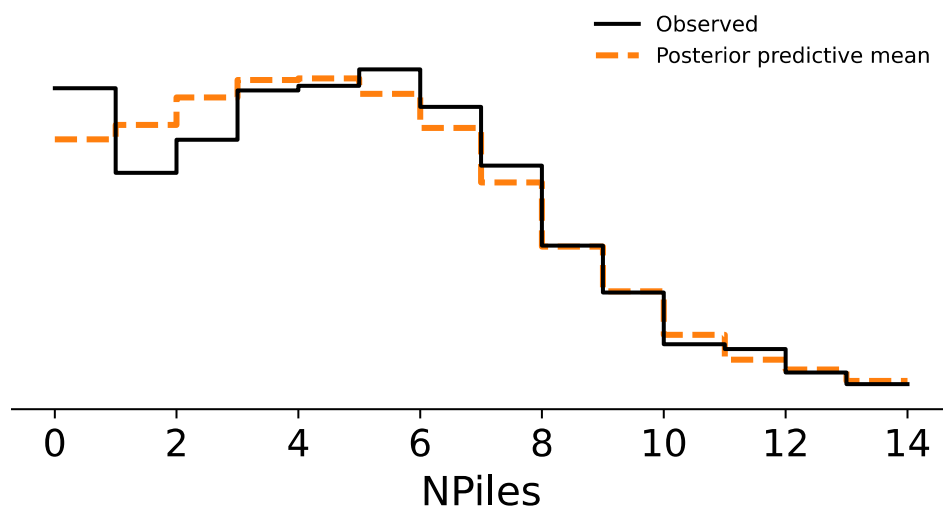


Figure 27 The posterior predictive probability for the number of piles an individual participates in

The black line is the distribution measured directly from the data.
The dashed orange line indicates the mean prediction of the model.

With confidence that the model does produce sensible results, the values and distributions of the free parameters can be used to infer the relative importance of genotype and phenotype on piling behaviour.

Figure 28 shows the effect size of each of these parameters on a log-odds scale. A value of zero indicates no effect on the number of piles an individual participates in. A positive (/negative) value indicates a positive (/negative) contribution to the number of piles.

When determining pile participation, the effect of the NO interaction time was greater than that of the sire (Figure 28). There is a variation in the contribution of sire, with a small number of sires having a negative contribution to piling with > 90% confidence (e.g. sires 5 and 25).

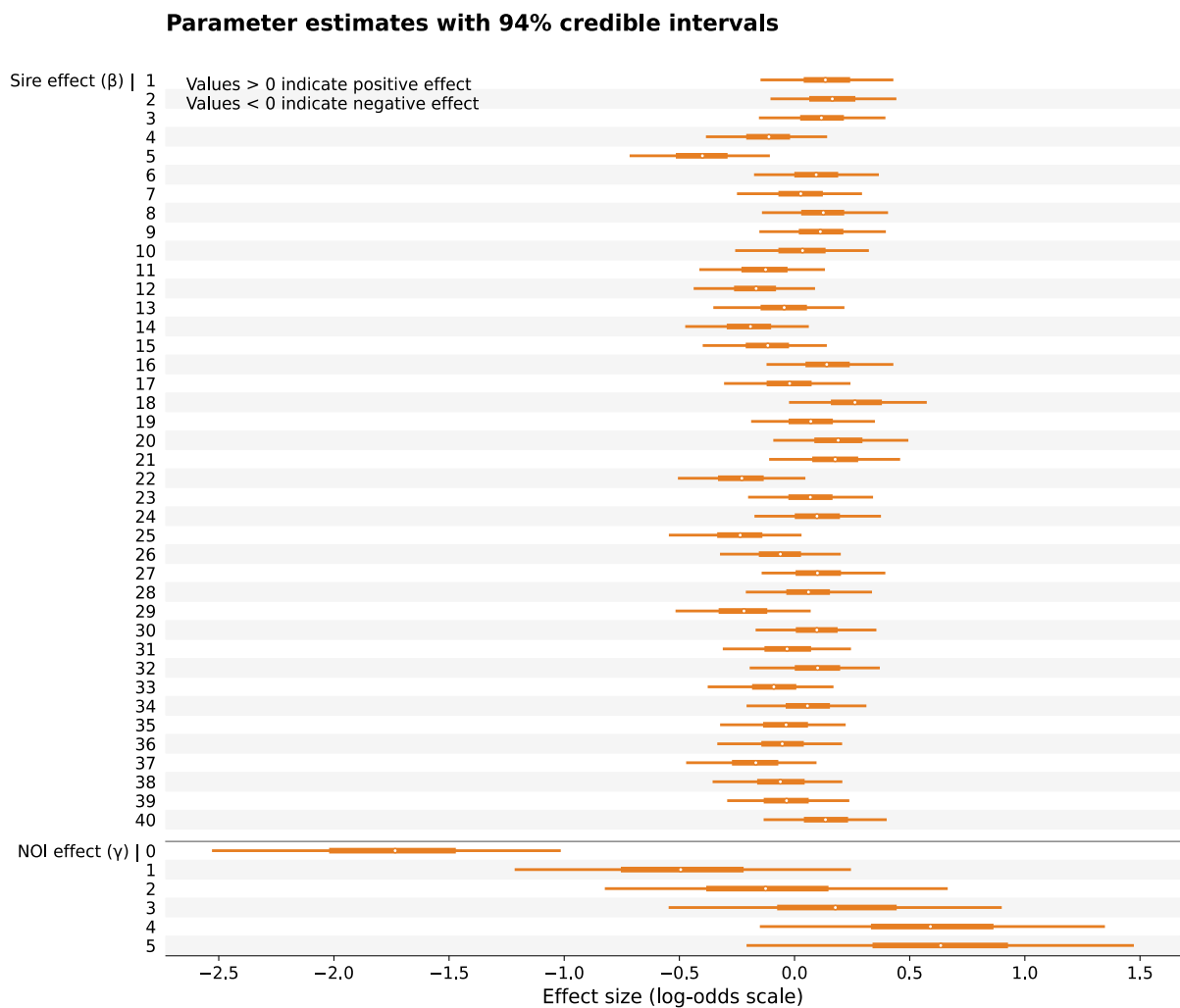


Figure 28 The distribution of effect parameters from our Bayesian generative model

The bars and lines indicate the 25–75 and 3–97 percentile ranges, respectively.

Rather than comparing the contributions of our model parameters directly, the relative importance of genotype and phenotype to piling behaviour can be further explored by comparing the support for different generative models, which include or exclude these effects. Figure 29 shows the expected log pointwise predictive density (ELPD) leave-one-out (LOO) scores for four different models. Higher scores represent more evidence to support that model, given the data. From this figure, we see that models that include the contribution of novel object interaction duration score better than those that do not (Figure 29).

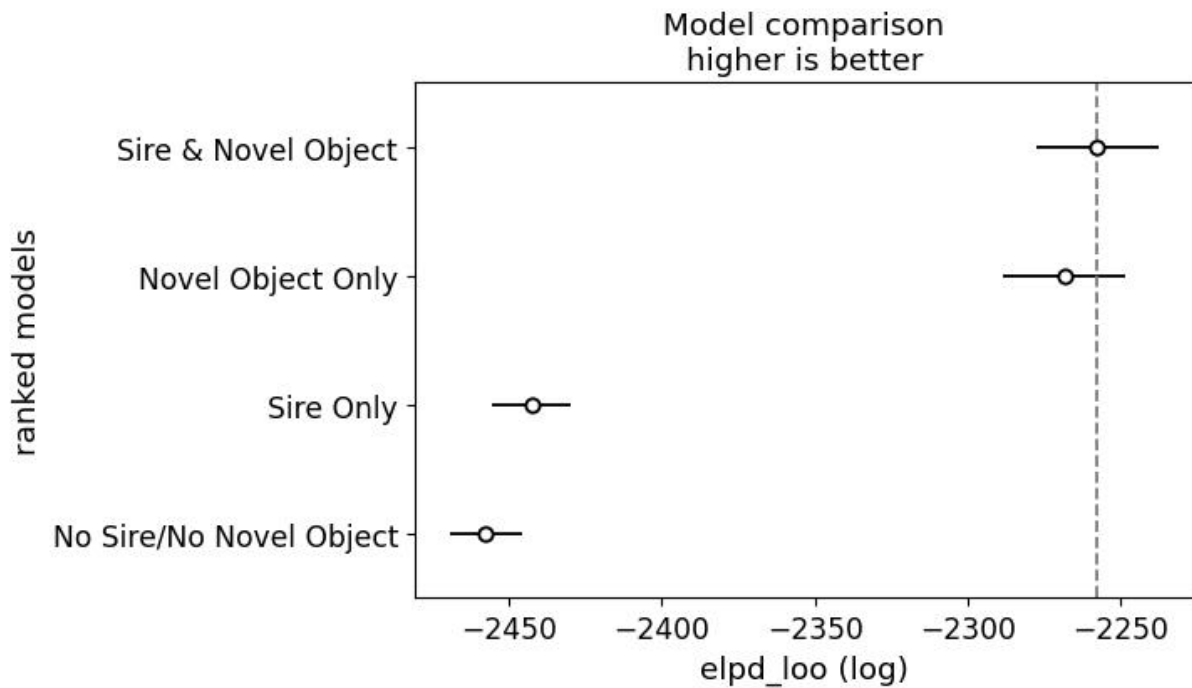


Figure 29 The expected log pointwise predictive density (ELPD) leave-one-out (LOO) scores for four different Bayesian models, which include or exclude the contribution of phenotype (novel object interaction) or genotype (sire) to piling behaviour

The effect of time spent interacting with the novel object evolves from negative to positive at our 6 different cut points (Figure 30). By plotting the log-odds effect as a function of novel object interaction duration, this can be seen clearly, as shown Figure 30. We also see that the effect of time spent interacting with the novel object for less than approximately 75 minutes (summed across all time points) decreased piling behaviour. However, longer interactions overall increased piling behaviour until reaching a plateau from 140 minutes onwards of interaction time with NOT.

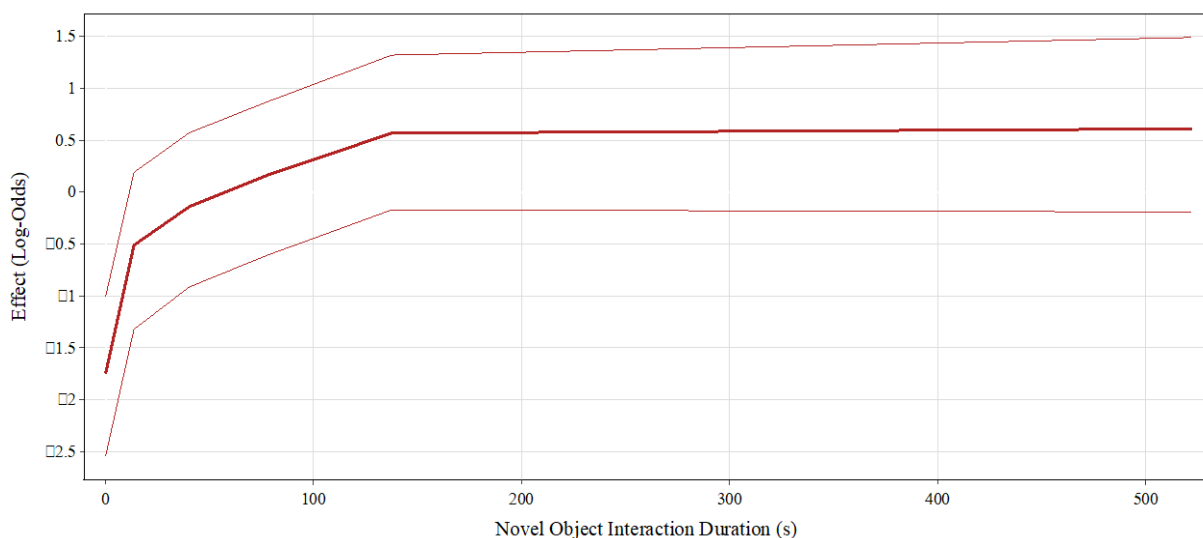


Figure 30 The effect of novel object interaction duration on piling behaviour as a function of interaction duration

The minimal spread that the sire introduces into the probability of participating in a pile is shown in Figure 31 and how the effect is dwarfed by behavioural phenotype (time spent interacting with the NO). Although the impact of sire was more pronounced at longer NO interactions (> 140 minutes), few hens contributed to that effect, as evidenced by the low mean time spent interacting with the NO (< 50 minutes; vertical dashed line in Figure 31).

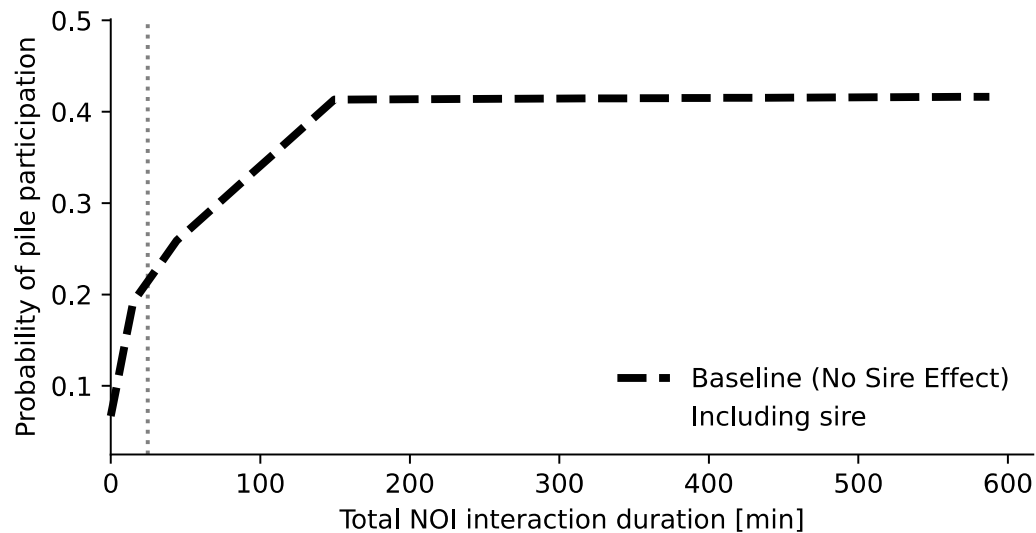


Figure 31 The mean probability of a hen participating in a pile as a function of total time spent interacting with the novel object (NO) (dashed line), derived from the best model fit (i.e. without sire)

The solid grey lines show the mean probability of participation in a pile as a function of time spent interacting with the NO split for each sire.

The vertical dotted line represents the mean time spent interacting with the NO.

4 Discussion

This study provides the first large-scale, longitudinal assessment of individual piling behaviour in group-housed laying hens across the production cycle. Piling increased in frequency, duration and participation with age and cumulative experience, but substantial and repeatable individual differences were evident. Behavioural phenotype, quantified through responses to repeated novel stimuli, was the strongest predictor of piling propensity, whereas genetics (sire) explained only a small proportion of variance. Together, these findings indicate that piling is a partly learned, socially reinforced behaviour that is strongly shaped by stable individual behavioural traits rather than genetics alone.

The hypothesis that piling behaviour is repeatable within individuals, across the production cycle, was supported. Across four observation periods, individual hens showed consistent tendencies to participate in piling events, accounting for approximately 10–15% of the variance in piling behaviour. A small but stable subset of hens never participated in piling, while others consistently joined early and remained in piles for longer durations. Repeatability estimates remained significant even after accounting for pen effects, location before pile formation and time point, indicating that individual differences were not solely driven by environmental opportunity. These findings align with the concept of stable behavioural phenotypes influencing collective behaviour, and suggest that individual motivation or social responsiveness plays a central role in piling dynamics, even within highly constrained group-housed environments.

The hypothesis that piling behaviour is heritable was weakly supported. Genetic background (sire) contributed a statistically significant but small proportion of variance (~1%) in piling behaviour. While differences between sire lines were detectable, they were dwarfed by individual-level variation and behavioural phenotype. These findings suggest that while genetics may influence susceptibility to piling, it is unlikely to be a strong or direct driver of recurring piling behaviour in isolation. This limited heritability is consistent with the low but non-zero heritability typical of complex behavioural traits (Van Oers et al. 2004) and aligns with preliminary findings in white strains (Stratmann A. 2023). These findings suggest that management and environmental interventions may be more effective than genetic selection alone in mitigating piling risk.

Behaviour phenotype predicted piling propensity, as hypothesised. Individual hens that interacted more with novel stimuli across the production cycle were more likely to participate in piling events. Bayesian modelling confirmed behavioural phenotype as the strongest predictor of piling propensity, exceeding the influence of sire or pen. Importantly, this relationship was not linear across all levels of interaction. Moderate levels of novel object interaction were associated with reduced piling, whereas very high interaction durations were associated with increased piling probability. This non-linear pattern suggests that rather than simple fearfulness or neophobia (Forkman et al. 2007; Hocking et al. 2001) piling may be linked to exploratory or socially responsive phenotypes.

These results contradict recent work by Chowdhury et al. (2025) who showed that flocks with a high fear of novelty (i.e. avoidance of an NO) were more at risk of smothering than flocks with less neophobia and more curiosity. This discrepancy may reflect differences in scale and methodology (i.e. flocks of ~225 hens compared to ~20,000 hens), but is also likely driven by differences in outcome measures, namely predicting smothering versus recurring piling. The piling observed in the current study was recurring piling, not piling associated with panic or nest box competition. It may be that the risk associated with fearfulness and smothering was reflective of panic smothering events, rather than smothering caused by recurring piling. Supporting this theory are findings by Rice (2023) who found that individual hens that were located close to a pile (detected by RFID technology) were faster to

approach a NO. Importantly, in the Rice et al. 2023 study piling hens were equally attracted to the 'short' and 'tall' NO, which may suggest that the relationships identified may rather have reflected hens' attraction to the cluster of hens around the NO, rather than the NO itself, as hens would have compromised visual contact with the short NO, relative to the tall.

The novel object test was implemented to characterise individual behavioural phenotype under commercial-relevant, group-housed conditions. Stimulus presentation was standardised in location across life stages to maximise within-individual comparability and reduce spatial confounds in large pens. However, different stimulus types were presented sequentially across time points, and birds were repeatedly exposed to novelty across the production cycle.

Consequently, time point effects reflect changes in responsiveness under a repeated exposure paradigm (i.e. age plus cumulative experience or habituation), rather than pure age-related differences in fearfulness. Responses to the novel object, therefore, represent context-specific behavioural tendencies rather than generalised fear of novelty. While this limits direct comparison with standardised single-object novelty tests, it provides ecologically relevant insight into stable individual differences in responsiveness within a commercial-style environment.

Future research should aim to replicate these findings across multiple flocks, housing systems and commercial environments to assess generalisability. Standardising stimulus-type across repeated measures, or explicitly manipulating stimulus salience, would help disentangle age, experience and object-specific effects on behavioural responses. Integrating automated tracking with real-time behavioural alerts may also enable early identification of piling-prone individuals or pens.

From an industry perspective, these findings suggest that recurring piling is unlikely to be driven by acute stress or fear alone. Instead, piling appears linked to behavioural phenotypes associated with exploration and social responsiveness. Interventions should therefore focus on managing how exploratory behaviour is expressed. For example, through environmental design or targeted dispersal strategies, rather than suppressing curiosity, which is also associated with positive welfare outcomes (Boissy et al. 2007).

5 References

- BOISSY, A., MANTEUFFEL, G., JENSEN, M. B., MOE, R. O., SPRUIJT, B., KEELING, L. J., WINCKLER, C., FORKMAN, B., DIMITROV, I. & LANGBEIN, J. 2007. Assessment of positive emotions in animals to improve their welfare. *Physiology & behavior*, 92, 375-397.
- BRIGHT, A. & JOHNSON, E. 2011. Smothering in commercial free-range laying hens: a preliminary investigation. *Anim. Behav.*, 119, 203-209.
- CHOWDHURY, P., HEMSWORTH, P., FISHER, A., RICE, M., GALEA, R., TAYLOR, P. & STEVENSON, M. 2024. Descriptive epidemiology of smothering in Australian commercial free-range layer hen farms. *Preventive Veterinary Medicine*, 223, 106098.
- CHOWDHURY, P., HEMSWORTH, P., FISHER, A., RICE, M., GALEA, R., TAYLOR, P. & STEVENSON, M. 2025. Risk factors for smothering in three commercial free-range layer poultry farms, Australia 2019 to 2022. *Preventive Veterinary Medicine*, 106568.
- DE HAAS, E. N., BOLHUIS, J. E., KEMP, B., GROOTHUIS, T. G. & RODENBURG, T. B. 2014. Parents and early life environment affect behavioral development of laying hen chickens. *PloS one*, 9, e90577.
- FORKMAN, B., BOISSY, A., MEUNIER-SALAÜN, M.-C., CANALI, E. & JONES, R. B. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & behavior*, 92, 340-374.
- GEBHARDT-HENRICH, S. G., KASHEV, A., PETELLE, M. B. & TOSCANO, M. J. 2023. Validation of a Radio frequency identification system for tracking location of laying hens in a quasi-commercial aviary system. *Peer Community Journal*, 3.
- GRAY, H., DAVIES, R., BRIGHT, A., RAYNER, A. & ASHER, L. 2020. Why do hens pile? Hypothesizing the causes and consequences. *Frontiers in Veterinary Science*, 7, 616836.
- HEDLUND, L., PALAZON, T. & JENSEN, P. 2021. Stress during commercial hatchery processing induces long-time negative cognitive judgement bias in chickens. *Animals*, 11, 1083.
- HERBERT, G. T., REDFEARN, W. D., BRASS, E., DALTON, H. A., GILL, R., BRASS, D., SMITH, C., RAYNER, A. C. & ASHER, L. 2021. Extreme crowding in laying hens during a recurrent smothering outbreak. *Veterinary Record*, 188, no-no.
- HOCKING, P., CHANNING, C., WADDINGTON, D. & JONES, R. 2001. Age-related changes in fear, sociality and pecking behaviours in two strains of laying hen. *British Poultry Science*, 42, 414-423.
- LARSEN, H., HEMSWORTH, P., CRONIN, G., GEBHARDT-HENRICH, S., SMITH, C. & RAULT, J.-L. 2018. Relationship between welfare and individual ranging behaviour in commercial free-range laying hens. *Animal*, 12, 2356-2364.
- MAZOCCO, C. C., DE CASTRO JÚNIOR, S. L., SILVEIRA, R. M. F., POLETO, R. & DA SILVA, I. J. O. 2024. Laying Hens: Why Smothering and Not Surviving?—A Literature Review. *Animals*, 14, 1518.
- PERINOT, E., PETELLE, M. B., GÓMEZ, Y. & TOSCANO, M. J. 2025. Temporal-spatial associations of large groups of laying hens in a quasi-commercial barn. *Applied Animal Behaviour Science*, 283, 106516.
- RICE, M., TAYLOR, P., HEMSWORTH, P., STRATMANN, A., TOSCANO, M.J. 2023. The individual assessment of responses of laying hens to a human stimulus and a novel object in relation to piling behaviour. *56th Congress of the International Society for Applied Ethology*. Tallinn.

- STADIG, L. M., AMPE, B. A., VAN GANSBEKE, S., VAN DEN BOGAERT, T., D'HAENENS, E., HEERKENS, J. L. & TUYTTENS, F. A. 2015. Opinion of Belgian egg farmers on hen welfare and its relationship with housing type. *Animals*, 6, 1.
- STRATMANN A., R. M., TOSCANO M.J. 2023. Piling characteristics of individual laying hens in small experimental flocks. *56th Congress of the International Society for Applied Ethology*. Tallinn
- VAN DER EIJK, J. A., RODENBURG, T. B., DE VRIES, H., KJAER, J. B., SMIDT, H., NAGUIB, M., KEMP, B. & LAMMERS, A. 2020. Early-life microbiota transplantation affects behavioural responses, serotonin and immune characteristics in chicken lines divergently selected on feather pecking. *Scientific Reports*, 10, 2750.
- VAN OERS, K., DRENT, P. J., DE GOEDE, P. & VAN NOORDWIJK, A. J. 2004. Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271, 65-73.
- WINTER, J., STRATMANN, A., TOSCANO, M. J., CARDWELL, J. M. & NICOL, C. J. 2022a. Piling behaviour in British layer flocks: Observations and farmersexperiences. *Applied Animal Behaviour Science*, 253, 105686.
- WINTER, J., TOSCANO, M. & STRATMANN, A. 2022b. The potential of a light spot, heat area, and novel object to attract laying hens and induce piling behaviour. *animal*, 16, 100567.

6 Plain English Summary

Project Title	Genotypic and phenotypic profiles of hens that pile
Australian Eggs Limited Project No.	INNO021
Researchers Involved	P.S. Taylor, A. Johnny, S. Mutch, R. Turnbull, A. Kashev, M. Fogarty and M.J. Toscano
Organisations Involved	Animal Welfare Science Centre, School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, The University of Melbourne, Parkville, Vic, Australia, 3010 Animal Welfare Science Centre, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Parkville, Vic, Australia, 3010 Centre for Proper Housing: Poultry and Rabbits, The University of Bern, Zollikofen, Switzerland.Melbourne Data Analytic Platform, The University of Melbourne, Parkville, Vic, Australia, 3010
Phone	+61 3 8344 9102
Fax	-
Email	peta.taylor@unimelb.edu.au
Objectives	This study aimed to: • measure how often individual hens pile over their lifetime, • determine if an individual's piling behaviour is consistent across the production cycle, • investigate whether piling is impacted by genetics, and • identify if hen temperament affects the likelihood of joining piles.
Background	Smothering is a serious welfare issue for laying hens, where hens crowd together so tightly that some die from suffocation. Smothering accounts for up to 16% of mortality in free range flocks in Australia. Smothering is caused by piling, a behaviour where hens cluster densely together, sometimes for long periods, with no obvious trigger. Not all piling leads to smothering, but all smothering starts with piling behaviour. What makes hens pile is largely unknown.
Research	Hens were observed for piling behaviour and their responses to novelty at four key stages in their life: before peak laying (20 weeks), during peak lay (30 weeks), after a commercially relevant stressor (52 weeks), and at the end of production (72 weeks). Individual hen behaviour was monitored using Radio Frequency identification technology throughout the aviary system. Across five pens, piling was common and increased in frequency and duration with age. About 50% of hens were involved in piling at least once, while around 10% never piled at all. Some hens joined piles earlier and stayed longer as they aged, suggesting they may learn or become more motivated to pile over time. Genetic differences (sire)

	explained a small part of the variation in piling, while individual behaviour explained much more. The strongest predictor of piling was how a hen responded to novelty, supporting a link between temperament and piling behaviour. Hens that were more fearful of novel objects were less likely to participate in piling events.
Outcomes	• Piling frequency increased with age and cumulative experience, with more hens participating in more piles for longer durations. • Piling behaviour varied consistently between individual hens. • A small number of hens never participated in piling, while others did so repeatedly. • Genetics played a minor role, but individual behaviour, particularly responses to novelty, was a strong predictor. • Piling appears to be shaped more by a hen's experience and personality than by inherited traits.
Implications	Understanding which hens are most likely to pile, and why, can help identify early warning signs and support better on-farm management. Since piling does not always lead to smothering but poses a clear risk, identifying the behavioural drivers of piling may reduce fatal outcomes. This knowledge can inform breeding, flock management, and housing design strategies to reduce piling and smothering and improve hen welfare.
Key Words	Smothering, Abnormal behaviour, Welfare