



Rearing enrichments differentially modified hen personality traits and reduced prediction of range use

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Domestic fowl have been demonstrated to individually differ in personality, dictating reactions to environmental stimuli. Free-range chickens, *Gallus gallus domesticus*, show individual range use variation, which may be related to personality differences. Enrichment can also modify behavioural traits. This study measured behavioural impacts of rearing enrichments across pullets and young adults, testing for personality differences and whether they predicted range use variation. Laying hens were reared for 16 weeks across nine pens under three conditions comparing control floor litter rearing with two enrichment types (novel objects or perching structures). At 16 weeks of age, 1386 pullets were transferred to a laying facility, housed in nine identical pens and given range access at 25 weeks. A sample of birds were tested at 9–11 weeks and 20–21 weeks with a series of tests: novel arena, adaptation to the arena with food present, open field, novel maze arena training with food and maze tests. Individual range use from 27 to 31 weeks was measured via radiofrequency identification technology. Enriched pullets were significantly quicker to first step in the adaptation with food present and structurally reared hens were significantly quicker to first step in the open field during testing, but there were no significant differences observed during maze arena training and testing. Of 16 correlations among test parameters, 11 were significant for control birds and six to seven were significant for the enriched treatments. Test parameter and range use correlations were significant for the control birds only. These results support the presence of different personalities in hens with enrichment reducing fear and increasing adaptation. Fewer correlations in the enriched hens suggest they developed a more plastic personality type. This response strategy may have fitness benefits for free-range birds or other captive-reared animals that undergo drastic environmental change across their lifetime.

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The assessment of personality within animal behaviour research is driven by the desire to understand how and why individuals consistently vary in their interactions with the environment they live in. Personality can dictate how an individual reacts to environmental and social stimuli and is typically defined as consistent among-individual behavioural differences across time (Biro & Stamps, 2008). Five categories of personality types have been defined in animals based on measures of activity, aggression, boldness/shyness, exploration and sociality (Réale, Reader, Sol, McDougall, & Dingemans, 2007). These are often assessed via measurements taken during individual behavioural tests such as open field, emergence, novel object, novel arena and social

attraction (Carter, Feeney, Marshall, Cowlshaw, & Heinsohn, 2013). Identifying different personalities among individuals provides researchers with a tool for understanding correlations between personality traits and growth or other life history traits (Biro & Stamps, 2008), personality traits and sociality (Webster & Ward, 2011), and personality traits and cognitive processes such as learning (Carere & Locurto, 2011). To date, different personalities have been confirmed across a wide range of species (Stamps & Groothuis, 2010), including domestic chickens, *Gallus gallus domesticus* (Garnham & Løvlie, 2018).

Personality testing with laboratory populations of red junglefowl, *Gallus gallus*, has provided evidence of individuality in attributes such as exploratory behaviour, activity and fearfulness (reviewed in Garnham & Løvlie, 2018), which may be related to performance across different testing dimensions, such as learning

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ability. However, in chickens, and across other species, the proximate and ultimate causes of these personality differences are currently not well understood (Carter et al., 2013). Laying hens show evidence of varying coping styles, passive and reactive, suggesting consistent differences among individuals in how they react to external stress-inducing stimuli (Cockrem, 2013). These stress-reactive personality differences have been shown to correlate with learning ability, where higher fear and sensitivity to stress can negatively impact subsequent learning test performance (de Haas, Hernandez et al., 2017; de Haas, Lee et al., 2017). However, not all studies are able to consistently categorize hens into these different coping styles (Van der Eijk, Lammers, Kjaer, & Rodenburg, 2019), and some research with junglefowl suggests personality traits are distinct from coping styles (Zidar, Balogh, et al., 2017). Thus, the relationship between personality and learning is complex; results show the relationship can also be dependent on the specific task as well as age at testing and bird sex (Zidar, Balogh, et al., 2018).

Personality traits show heritability (Drent, van Oers, & van Noordwijk, 2003) but are also shaped during ontogeny (Groothuis & Trillmich, 2011), which may be related to different life stages and hormonal change with sexual maturity (Stamps & Groothuis, 2010). In junglefowl, personality changes throughout the development of the bird with limited prediction of adult personality from assessments made earlier in life (Favati, Zidar, Thorpe, Jensen, & Løvlie, 2015). Furthermore, early life experiences can modify personality traits in adults. For example, cognitive stimulation (or not) of junglefowl chicks affected the personality traits of the same birds as adults (Zidar, Sorato, et al., 2017). Personalities that change across an animal's lifetime may be reflective of an individual's adaptive capacity in response to changing environmental surroundings (Groothuis & Carere, 2005; Trillmich, Günther, Müller, Reinhold, & Sachser, 2015). This effect of ontogenetic experiences on interindividual behavioural plasticity is currently less well understood (Stamps, 2016).

Environmental enrichment is a type of environmental manipulation that has been demonstrated to affect measures of personality traits in chickens. Provision of enrichment for chickens has reduced fear- and stress-related responses as well as cognitive biases (in some cases), indicating positive impact of enriched developmental or adult housing conditions on behavioural traits (Jones & Waddington, 1992; Ross et al., 2019, 2020; Zidar, Campderrich, et al., 2018) and coping styles (Campbell, Hinch, Downing, & Lee, 2018). Rearing with environmental complexity also improves later performance in spatial cognition tasks (Norman, Adriaense, & Nicol, 2019; Tahamtani, Nordgreen, Nordquist, & Janczak, 2015). However, the extent to which enrichments may affect the development of personality in chickens is unclear.

Evidence for individual personalities in chickens has been demonstrated (Garnham & Løvlie, 2018), and domesticated laying hens in production housing environments show high individual variation in how they utilize the areas and resources available to them, such as different sections of indoor aviary systems (Campbell, Karcher, & Siegford, 2016; Rufener et al., 2018) and, more notably, the indoor and outdoor areas of free-range systems. Individual hens may range daily for many hours, daily for only a few hours or prefer to remain inside (reviewed in Campbell, Bari, & Rault, 2021). This individual variation is consistently present across different flocks and hen strains and in both commercial and experimental settings (Campbell et al., 2020). The variation in resource use among hens of similar genetic background may be related to individual variation in personality. While they may not be measures of personality per se (i.e. not conducted across time), behavioural tests conducted across multiple separate studies show, overall, that hens who remain inside or exhibit lower ranging also show increased fear and anxiety and reduced boldness, although not every test conducted

has detected differences among individuals of varying range use patterns (Campbell, Hinch, Downing, & Lee, 2016; Campbell, Dickson et al., 2019; Campbell, Taylor et al., 2019; Hartcher et al., 2016; Larsen et al., 2018).

Variation in outdoor ranging in meat chickens is also demonstrated to correlate with performance in cognitive tests, providing further evidence for consistent differences related to range use behaviour. Birds that visited the range less often were faster to reach training criterion in a spatial memory task and showed decreased latencies along with improved memory performance compared with chickens that visited the range more often (Ferreira et al., 2019). Similarly, these low-ranging birds showed better spatial memory performance when they had to find the reward based on more complex distal (cf. local) cues (Ferreira, Barbarat, et al., 2020). The high-ranging birds also showed poorer performance in a detour-reaching task, indicating they had poorer motor self-regulation compared with the low-ranging birds (Ferreira, Reiter, Germain, Calandreau, & Guesdon, 2020). However, these described behavioural and cognitive tests were conducted after ranging patterns among individuals were already established and this may have influenced the test results. Alternatively, in some predictive testing, time spent close to conspecifics in a social motivation test was negatively correlated with subsequent range use, but variables assessed in open field and emergence tests showed no relationship with ranging (Ferreira, Barbarat, et al., 2020). In a maze learning task with laying hens, faster performance speed was positively correlated with subsequent numbers of daily visits to the range, but only in pullets that had been exposed to enrichment during the first 3 weeks of life (Campbell, Talk, Loh, Dyall, & Lee, 2018). Thus, the distinction between pre-existing personality differences predicting ranging behaviour versus ranging behaviour modifying personality is unclear.

In the present study, we applied multiple behavioural tests to hens reared under different enriched environments to determine whether measurements of fear and anxiety would be consistent within individuals across time and affected by rearing condition, whether these measurements would be correlated with performance in a maze test and whether behavioural indices would be correlated with subsequent individual variation in range access. We predicted that rearing enrichments would reduce pullet and hen fear, improve spatial cognitive performance and decrease personality consistency and that learning and boldness would be correlated with range use during the initial ranging period.

METHODS

Ethical Statement

The research was approved by the University of New England Animal Ethics Committee (AEC17-092). Housing resources were provided in accordance with the Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002) with all birds inspected daily to ensure immediate detection of any visible welfare issues. Trained personnel conducted all testing to ensure careful handling of the birds, and all birds were immediately returned to their home pens following the short behavioural tests conducted in these trials. Some behavioural tests may have resulted in a stress response for some/all birds, but this response was anticipated to be short-lived as testing duration was not prolonged. All testing procedures were noninvasive.

Animals and Pullet Housing

The first part of the study was conducted in the Rob Cumming Poultry Innovation Centre of the University of New England,

Armidale, Australia, and birds were part of the larger overall study assessing the effects of rearing enrichments on laying hen behaviour, health and welfare (Bari, Downing, Dyall, Lee, & Campbell, 2020; Campbell, Dyall, Downing, Cohen-Barnhouse, & Lee, 2020). Hy-Line Brown day-old layer chicks ($N = 1700$) were housed indoors within nine floor pens (6.2×3.2 m) across three separate rooms. Each pen included rice hulls as floor litter, water nipples and round feeders provided in accordance with the current Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002). Mash formulated for specific growth stages and water were provided ad libitum. Across 16 weeks, pullets were reared under three different enrichment treatments with one treatment replicate in each room, balanced for location. Shade cloth on the wire pen dividers visually isolated birds among the treatments. The treatments included (1) a 'control' treatment with no additional enrichment, (2) a 'novelty' treatment where various novel objects were exchanged approximately weekly including balls, bottles, brooms, buckets, disks, ropes, chain, cinder blocks, containers, dog toys, milk jugs, plastic kids toys, pipes and strings and (3) a 'structural' treatment, which included five custom-designed perching apparatuses made of painted black metal and coated in a nonslip covering ($0.6 \times 0.6 \times 0.6$ m). Each structure had two solid sides and an open-framed side forming an H-shaped structure that could be placed in different orientations. Pullets could perch on the structures, but the solid side in some orientations would provide a visual/physical obstruction requiring navigation around it. The bird density at 16 weeks was approximately 15 kg/m^2 or nine pullets/ m^2 , with a total of 174–190 birds per pen (variation among pens resulted from both chick mortality and placement error). Each room was mechanically ventilated with heating and lighting schedules following the Hy-Line Brown recommendations for alternative system management (Hy-Line, 2016). However, the artificial LED lighting (no natural light) was maintained at 100 lx as the pullets were being reared for outdoor access. All chicks and pullets were vaccinated as per regulatory requirements and standard recommendations for the region.

Novel Arena Test

We carried out a series of tests on individual birds at 9–21 weeks of age as per the timeline schematic in Fig. 1. Testing started at 9 weeks as brain synapses continue maturing until around 8–10 weeks of age; thus, testing around this age is hypothesized to allow time for impacts of the rearing treatments (Rogers, 1995). The hens in the overall flock started coming into lay at 18 weeks of age (Bari, Cohen-Barnhouse, & Campbell, 2020) and thus the later testing was conducted as the hens were coming into (or were already at) sexual maturity. A clear reduction in fear is typically observed as the birds come into sexual maturity (Campbell, Talk, et al., 2018). We first conducted a novel arena test in a separate room at the same facility on 87 randomly selected pullets ($N = 29$ control, $N = 28$ novelty,

$N = 30$ structural hens) at 9 weeks of age. The sample sizes per treatment varied based on exclusion of some pullets from the data set that were not tested in subsequent tests as adults as they were unable to be located in the home pens at the time of testing. The wooden, rectangular ($2.4 \times 0.8 \times 0.8$ m) novel arena was placed on a cement floor and covered with wire mesh to prevent bird escape. The arena contained four zones divided by metal framework (that laid flat on the floor and held the wire mesh roof in place), with the first zone having a sliding door that could hold the birds in that area (holding zone). The holding zone was 42 cm, with the remaining zones 67 cm in length each (Fig. 2a). Pullets were individually caught from their home pen and carried to the new room with a towel over their head to shield their eyes as birds had to be taken outside before entering the testing room. They were placed in the holding zone for 20 s before the removable wooden separator door was slid open. This 20 s time period was selected to both allow the hens time to adjust body position following their placement into the arena and for the experimenters to set the camera recording for each bird. An adjustment period was not a direct part of the behavioural test as the test aimed to measure immediate responses of the birds to the novel arena. The bird had free access to the arena for a 10 min period before the test concluded and the pullet was returned to the home pen. Each pullet was fitted with unique numbered and coloured leg bands at the conclusion of their test. All experimenters were out of sight but within the room during testing. Testing order was balanced across pens and treatments and occurred across a 3-day period from approximately 0800 until 1800 hours. A trained observer in the room recorded latency to first vocalize and all vocalizations made during the test by counting each individual sound the pullet produced (different call types were not distinguished) and was not blind to the rearing treatments of the pullets. The behaviour of each bird was also videorecorded (Sony HDR-PJ410 Handycam, Sony Corporation, Tokyo, Japan) and later observed by a single experimenter blinded to the rearing treatment to count the latency to first step, latency to first enter the final zone (two feet over the metal divider), the total number of steps and number of escape attempts (flying up into the wire mesh).

Adaptation to the Novel Arena with Food

Following the initial test in the novel arena, the same pullets were tested for acclimation to the arena and willingness to eat over two separate testing sessions occurring on different days. For these two adaptation tests, the test area was reduced by placing a sliding door to exclude the last zone (zone 4; see Fig. 2a) and a clear bowl of poultry mixed grain and live mealworms, *Tenebrio molitor*, was added at the end of the arena (at the end of zone 3). The test area was reduced following observations during the novel arena test that few birds were moving to the end of the arena. The smaller test area was hypothesized to increase the chance that birds may approach the feed. Prior to the adaptation trials, birds were exposed to the grain in their home pens across 2 days by adding it to their

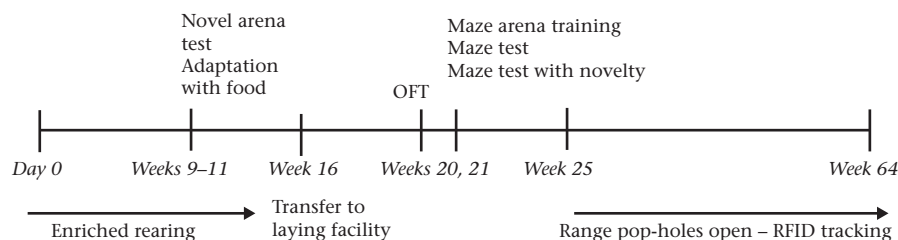


Figure 1. Trial testing timeline. A schematic of the tests (OFT: open field test) conducted on the pullets and laying hens at different age points including the transfer to the laying facility and pop-hole opening to allow range access, which was tracked via radiofrequency identification (RFID) technology.

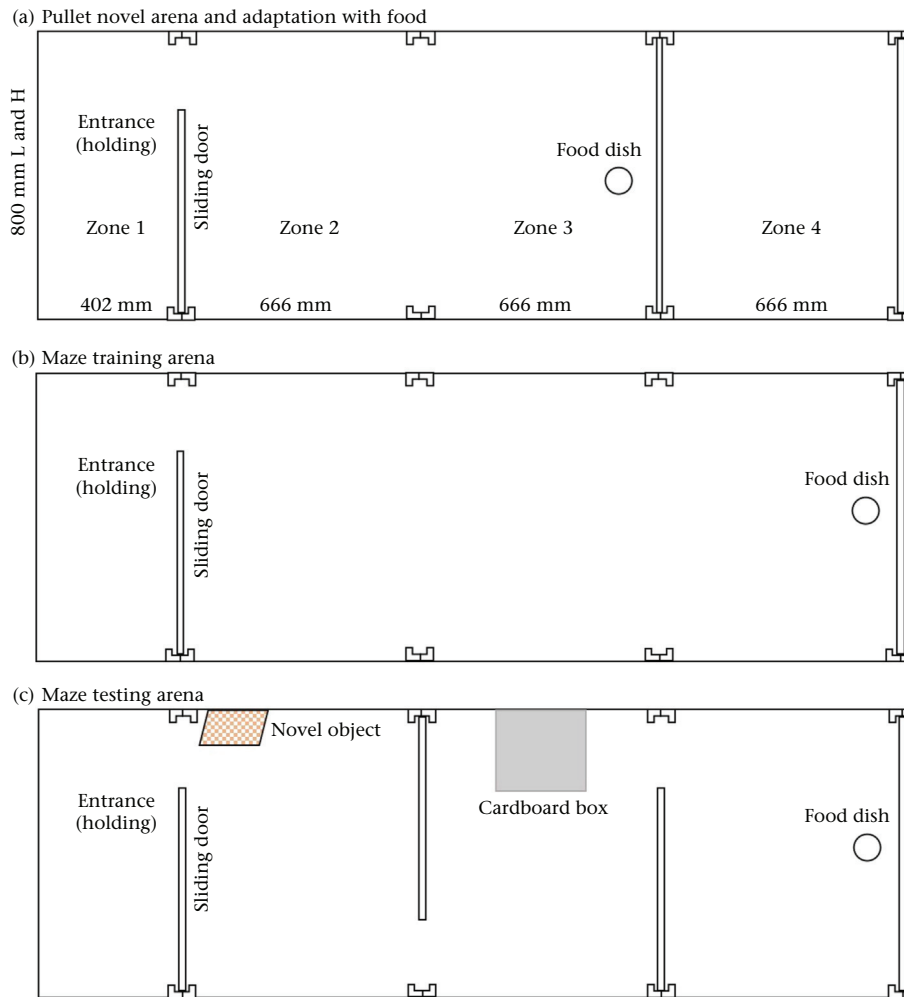


Figure 2. (a–c) Varying configurations of the novel test arena. The same arena was used for several tests in pullets and hens but was modified by sliding panels, addition of objects and presence/absence of food. For each test, birds were initially held in the holding zone (zone 1) before the door was slid open (it opened fully in 'a' and 'b' but only partially in 'c'). The arena (a) was used with all zones available in the novel arena test (see Novel Arena Test), then the final zone (zone 4) was closed off and food was added at the end of zone 3 for the adaptation trials (see Adaptation to the Novel Arena with Food). The same arena was used for training to reach the food dish and eat (b) as described in Stage 1: Arena training. The maze test (c) was then conducted twice with the novel object (patterned, coloured fabric) lowered over the side of the maze by the exit opening in the final test. Birds had to complete the maze to reach the food dish. The arena was covered with wire mesh to prevent the birds from escaping.

feeders and placing some on the litter. However, it could not be verified whether each tested pullet consumed this grain. There were insufficient mealworms to acclimate birds to these in the home pens. Starting at 9 weeks of age, the same pullets were caught from their home pens, carried to the testing room with a towel over their head and placed into the holding zone for 20 s. The door was slid open and the hen was able to explore the arena for 10 min. A trained observer (not blind to the rearing treatments) recorded the latency to first vocalize and the behaviour of the bird was video-recorded (Sony HDR-PJ410 Handycam). At the conclusion of the test the individual bird was removed from the arena and returned to its home pen. Additional leg bands were added to identify tested birds. Testing was carried out from approximately 0900 until 1700 hours (some days were of shorter duration) across a period of 5 days, with bird selection across the day balanced across pens and rearing treatments. When each pullet had been tested across one adaptation session, all birds were tested for a second adaptation session across a period of 5 days (totalling three 10 min test periods in the wooden arena per bird from 9 until 11 weeks of age). Although the exact testing order of individual birds was not repeated across the novel arena and adaptation sessions due to the logistics of identifying specific band numbers of each tagged subject bird within the

pen, a similar testing order was followed so that the intertest interval per bird was matched as best as logistically possible in terms of days. A trained observer (blinded to the rearing treatments) later watched the videos to record the latency to first step, latency to enter zone 3 (both feet over the metal divider), latency to eat and number of escape attempts.

Layer Housing

At 16 weeks of age all remaining pullets were transported to the layer facility ($N = 1386$, some pullets that were of lower or higher body weight, or randomly selected, were rehomed as they were surplus to the capacity of the layer shed). Birds were socially remixed within their treatment pen replicates and placed into nine identical pens (4.8×3.6 m, visually isolated by shade cloth) within a single shed. Three pen replicates per treatment were balanced for location across the shed. Each pen had two suspended round feeders, water nipples (single water line), perches and nestboxes with rice hulls as the floor substrate (see Campbell et al., 2020 for a pen schematic). The pen resources were provided to meet the Australian Model Code of Practice for the Welfare of Animals – Domestic Poultry (Primary Industries Standing Committee, 2002)

with the perching space at 10 cm per bird due to logistical space restrictions within the pen (birds also perched on the waterlines and feeders). Commercial mash formulated for production stages was provided *ad libitum*. The LED lighting schedule was set at 10:14 h light:dark, with mean light intensity at $10.0 (\pm 0.84 \text{ SE}) \text{ lx}$ (Lutron Light Meter, LX-112850; Lutron Electronic Enterprise CO., Ltd, Taipei, Taiwan) as measured at the birds' eye height from three locations (front, middle, back) within each pen (the brightest bulbs allowed by the shed wiring system). The shed was fan-ventilated with no other environmental controls.

Open Field Test

At 20 weeks of age (Fig. 1), the same 87 hens that were tested as pullets were assessed in an open field test in a separate room within the same laying hen facility across a period of 4 days from approximately 0900 until 1600 hours. The square wooden open field box ($1.25 \times 1.22 \text{ m}$, elevated 0.24 m off the ground) had an opaque roof, three opaque side panels and a clear frontal Perspex sliding panel. Banded hens were caught from their home pens as they were found and immediately carried to the testing room. Order of tested birds was balanced across pen replicates and treatments. Each bird was placed in the centre of the box in the dark, lights were then turned on and the test began. All birds were viewed on a monitor during testing by an experimenter out of sight but present in the room. All behaviour during testing was also videorecorded (Sony HDR-PJ410 Handycam). The latency to first vocalize and all vocalizations produced were recorded by a trained observer (not blind to the rearing treatments), with each sound counted as one vocalization (different call types were not distinguished). After 5 min, the test concluded, the lights were turned off and the bird was returned to its home pen. Later, a single trained observer viewed all video to record the latency to first step and total number of steps made. Birds that did not perform a specific behaviour were assigned a maximum latency equal to the test duration.

Maze Test

At 21 weeks of age (Fig. 1), the same pullets ($N = 87$) were trained in the same arena that was used in the first novel arena test (see Novel Arena Test). They were then tested in the same arena but with modifications to convert the open area into a maze (Fig. 2b and c). The maze was placed in a corner of the same shed that the birds were housed in. During testing, birds were visually but not acoustically isolated from their flockmates. A series of cameras (Sony HDR-PJ410 Handycam) recorded all hen behaviour in the maze and additional cameras were connected to monitors to enable an observer to view the hens without being seen. Birds were individually caught from their home pens as they were identified, with order of bird selection balanced across pen replicates and treatments. Approximately 27 hens could be trained on a single day. Training and testing occurred across two separate days and were sequential for each hen. All hens were trained and tested across a period of 7 days.

Stage 1: arena training

All birds were initially trained to approach and consume food located at the end of the arena. The transparent plastic food dish had *ad libitum* mealworms placed on top of a pile of poultry-mixed grain. The birds had been given grain as pullets during the adaptation sessions (see Adaptation to the Novel Arena with Food) and were also provided grain, but not mealworms (as there were not sufficient quantities of worms to provide them in the home pens for 1386 hens), in their home pens across 2 days directly prior to the

maze training. The mixed grain was provided to visually encourage the hens to approach the mealworms, but mealworms were preferred once birds had consumed them. Some birds may have consumed mealworms as pullets, but the precise number was uncertain from the top-down video recording during the adaptation sessions. Training for each bird occurred across 2 days at 21 weeks of age with eight training sessions per bird (five consecutive sessions on day 1, followed by three consecutive sessions on day 2 as described in Stage 2: maze testing). The test bird was initially placed in the closed holding zone for 30 s, then the door was slid completely open and the training session commenced. This 30 s period allowed the bird to adjust its body position, as needed, following placement and allowed the single experimenter sufficient time to prepare the video recording for each test session (bird identity had to be written on a card to hold in front of the cameras). There were no barriers between the bird and food located at the end of the maze arena (zone 4; Fig. 2b). The hens were permitted up to 5 min to approach and consume food. The training session concluded once at least one mealworm had been consumed, and this latency was recorded. If a bird did not approach the food, it was assigned a maximum latency of 300 s, then manually placed ($N = 15/87$) in front of the food and gently held in location for a few seconds until it was clear the bird had seen the mealworms (the Hy-Line Brown hens used in the study typically did not struggle when held). If the hen did not peck at the mealworms, then we placed some mealworms on the ground in front of the hen, as the movement of the mealworms attracted the attention of the hen and stimulated pecking. Once the hen had consumed at least one mealworm, she was gently pushed back into the holding zone using a clipboard. Some hens did not eat during this first training session ($N = 10/87$). The holding zone was closed for another 30 s and the door was opened to let the hen out. We permitted the hen up to 3 min to approach and consume the food for the second training session and recorded her latency to consume the food. If a hen did not approach after 3 min, then we manually placed her at the food again and held her there for a few seconds. This was necessary for nine hens. Hens were gently pushed back into the holding zone once they had consumed at least one mealworm. The immediately consecutive training sessions 3, 4 and 5 followed the same protocol with only six hens needing to be placed at training session 3. All hens were readily approaching and consuming the feed during training session 5 except for seven hens that were unable to be trained (did not approach and readily consume the food; control: $N = 2$, novelty: $N = 5$) and these were excluded from any subsequent testing.

Stage 2: maze testing

We tested 80 pullets in the second stage, which was designed as a test of spatial learning as well as boldness when the novel object was introduced. However, the first four hens were used to test a modified maze set-up once it was apparent that the first maze design (without the cardboard box shown in Fig. 2c) was too simple. Thus, a final total of 76 hens (control: $N = 24$, novelty: $N = 23$, structural: $N = 29$) were tested throughout stage 2. The day following the first five training sessions, individual hens were placed back into the holding zone for 30 s. We permitted them access to the food at the end of the arena and recorded latency to approach and consume across three consecutive sessions. A maximum of 300 s was allotted for each training session, but no hens reached that maximum time. For the fourth session on that day (ninth session in total across the 2 days), the arena was turned into a maze (Fig. 2c) where the hens had to navigate past three panels and a cardboard box to reach the food. The door to the holding zone was also only partially slid open wide enough to allow the bird to exit. We recorded the latency to leave the holding zone

(2 feet past the sliding door), latency to eat as well as the number of errors. An error was categorized as movement back towards the holding zone rather than continuing on to reach the food. Hens were permitted up to 5 min to reach and eat the food and were then gently pushed back through the maze into the holding zone. Following 30 s in the holding zone, the hens were presented with the same maze configuration a second time, but a novel object was lowered over the side of the maze right at the entrance door (Fig. 2c). This was a piece of coloured and patterned fabric on the left-hand side as the birds exited. We recorded their latency to leave the holding zone (2 feet past the sliding door) as well as their latency to eat. A maximum of 5 min was allotted to complete the maze in the presence of the novel object distraction. Six hens flew out of the holding zone when the novel object was present, and we included their latency until escape in the data as their latency to leave the holding zone with the maximum test time (300 s) assigned to their latency to eat.

Radiofrequency identification (RFID) system and data

Before transfer to the laying facility, all hens were banded with microchips (Trovan Unique ID 100, FDX-A, operating frequency 128 kHz, RFID Systems Ltd, North Ferriby, U.K.) glued into adjustable leg bands (Roxan Developments Ltd, Selkirk, U.K.). Radiofrequency identification (RFID) systems were set up in the indoor pens (Campbell et al., 2017). The RFID systems were designed and supported by Microchips Australia Pty Ltd (Keysborough, VIC, Australia) with equipment developed and manufactured by Dorset Identification B.V. (Aalten, The Netherlands) using Trovan technology (RFID Systems Ltd). Hens could access the range via two pop-holes per pen (18 × 36 cm) that contained antennas, allowing us to track the range use movement of individual hens. The RFID system recorded the date and time of each banded bird passing through the pop-hole and in which direction (onto the range or into the pen) with a precision of 0.024 s (maximum detection velocity 9.3 m/s). The automated pop-holes were first opened when birds were 25 weeks old and individual ranging data were collected daily from 0915 hours until after sunset (approximately 9 h of available range access, then approximately 11 h once Daylight Saving Time started before the later ranging period). We used daily range use data from weeks 27–31 (24 days) as few hens ventured outside when the pop-holes were first opened (see Campbell et al., 2020 for a full report on range access). We also selected 24 days of ranging data at the end of the trial (62–64 weeks of age) for comparison with initial range use. If a hen did not access the range, then no data were recorded. The daily data were run through a custom-designed software program written in the 'Delphi' language (Bryce Little, CSIRO, Agriculture and Food, St Lucia, QLD, Australia) that filtered out any unpaired or 'false' readings that occurred if, for example, a hen did not complete a full transition onto the range or back into the pen. The same program summarized the daily data to provide an average of time spent outside, average number of visits to the range and the total proportion of available days the range was accessed per individual hen across the collection period.

Data and Statistical Analyses

All analyses were conducted in JMP 14.0 (SAS Institute, Cary, NC, U.S.A.) with α set at 0.05. Data transformations were applied where needed for parametric tests with the Studentized residuals visually inspected for homoscedasticity. Raw data are displayed in the tables and figures. Birds that failed to perform specific behaviours within the maximum test time were censored results for the survival analyses.

Data from the novel arena test were compiled per bird to include the latency to first vocalize, latency to first step, latency to reach

zone 4, number of vocalizations, number of steps and number of escape attempts. The latencies to first step and to vocalize were analysed using separate Kaplan–Meier estimates (Jahn-Eimermacher, Lasarzik, & Raber, 2011) with a log-rank test for differences between rearing treatment groups. The latencies to reach zone 4 were analysed using Kruskal–Wallis tests for nonparametric data as the values for 41 birds were censored (i.e. birds did not perform the behaviour). The number of steps and number of vocalizations were square-root transformed and analysed using general linear mixed models with the fixed effect of rearing treatment including pen as a random effect. Restricted maximum likelihood estimation (REML) methods were applied. The number of escape attempts were analysed using a Kruskal–Wallis test for nonparametric data due to few escape occurrences.

Data from the adaptation sessions with food present were compiled per bird to include the latencies to first step, vocalize, reach the feed zone, and to eat. The latencies to first step, to vocalize and to reach the feed zone were analysed using separate Kaplan–Meier estimates per rearing treatment with a log-rank test for differences between adaptation sessions. Data for the latency to feed were analysed using separate nonparametric Wilcoxon signed-rank tests per rearing treatment as the data were not normal and 60% and 45% of birds did not eat across the two adaptation sessions, respectively.

Data from the open field test were compiled per bird to include the latency to first vocalize, latency to first step, number of vocalizations and number of steps. The latencies were analysed using separate Kaplan–Meier estimates with a log-rank test for differences between rearing treatment groups. The count data were square-root transformed and analysed using general linear mixed models with the fixed effect of treatment and pen included as a random effect.

We compiled the summed latency to eat data for the first five training sessions and the three training sessions the following day per bird as well as the latency to eat when the maze was present and analysed the data using separate Kaplan–Meier estimates with a log-rank test for differences between rearing treatment groups. The number of errors made during the maze test per bird were analysed using a Kruskal–Wallis test. Latency to leave the holding zone during the maze test was compiled per bird and analysed using a Kaplan–Meier estimate with a log-rank test for differences between rearing treatment groups. The data for latencies to leave the holding zone and to eat in the presence of the novel object were analysed using separate Kruskal–Wallis tests due to 31 and 38 values being censored, respectively.

To assess consistency in behavioural responses by individual birds across time, we applied Spearman's pairwise correlations separately for birds from each rearing treatment to the latency values between the different tests across time. Latencies were compared among values that were collected across time for either separate tests or separate days within the same test. If latency values within the same test on the same day were highly correlated, only one was selected. In total, we made 16 comparisons between latency values.

Four latency values were selected to correlate with the mean daily time outside and mean daily visits from the RFID data collected during the early ranging period. The selected values were predicted to be indicative of range usage based on previous research (Campbell, Talk, et al., 2018; Ferreira et al., 2019; Ferreira, Barbarat, et al., 2020; Larsen et al., 2018): latency to vocalize in the open field test, the summed latency to eat across maze arena training sessions 2–5, the latency to eat in the maze test and the latency to leave the holding zone when the novel object was present in the maze. Spearman's correlations were also applied

between the early range use data and the late range use data separately for each rearing treatment.

RESULTS

Novel Arena Test and Adaptation Sessions

There were no significant effects of rearing treatment on any of the behavioural parameters assessed in the novel arena test (all $P \geq 0.29$; Table 1). While the pullets did vocalize and step, they took the majority of the testing time to reach the last zone of the arena (Table 1).

The Kaplan–Meier estimates showed a significant difference between the two adaptation sessions with food present in the latency to step for the novelty ($\chi^2_1 = 6.70$, $P = 0.01$) and structural ($\chi^2_1 = 7.74$, $P = 0.005$) treatment groups, but only a nonsignificant tendency for the control birds ($\chi^2_1 = 3.21$, $P = 0.07$). The enriched-reared pullets were quicker to step in their second adaptation session (both $P \leq 0.01$), but the control birds only showed a nonsignificant tendency to be quicker ($P = 0.07$; Table 2). The novelty hens were quicker to vocalize in their second adaptation session ($P = 0.04$), and the structural ($P = 0.07$) and control ($P = 0.09$) birds showed a nonsignificant tendency to be quicker (Table 2). The structural birds showed a significantly faster latency to reach the feed zone in their second adaptation session ($P = 0.03$), but the novelty ($P = 0.08$) and control ($P = 0.09$) birds only showed a nonsignificant tendency to be quicker. In contrast, the novelty birds were significantly quicker to eat in the second adaptation session ($P = 0.04$), whereas the control ($P = 0.10$) and structural ($P = 0.26$) birds did not show significant differences (Table 2). Overall, only 35 of 87 birds ate in the first adaptation session (control: $N = 11$, novelty: $N = 13$, structural: $N = 11$) and this increased to 47 birds in the second session (control: $N = 16$, novelty: $N = 18$, structural: $N = 13$).

Open Field Test

The Kaplan–Meier estimates showed an effect of rearing treatment on the latency to first step ($\chi^2_2 = 19.86$, $P < 0.0001$; mean \pm SD: control: 175.39 ± 108.32 [95% CI 133.4, 217.4]; novelty: 131.41 ± 95.02 [95% CI 95.3, 167.6]; structural: 77.63 ± 50.70 [95% CI 58.7, 96.6]) and latency to vocalize ($\chi^2_2 = 6.68$, $P = 0.04$; mean \pm SD: control: 87.68 ± 107.67 [95% CI 45.9, 129.4]; novelty: 66.24 ± 80.46 [95% CI 35.6, 96.8]; structural: 34.9 ± 36.30 [95% CI 21.3, 48.5]), with the structural hens showing shorter latencies for both behaviours (Fig. 3). But there were no significant differences between treatments in the number of steps ($F_{2,5,97} = 2.52$, $P = 0.16$; mean \pm SD: control: 31.6 ± 35.0 [95% CI 18.1, 45.2]; novelty: 35.0 ± 28.0 [95% CI 24.4, 45.7]; structural: 50.1 ± 26.9 [95% CI 40.0, 60.1]), or number of vocalizations ($F_{2,4,21} = 1.73$, $P = 0.28$; mean \pm SD: control: 65.1 ± 67.9 [95% CI 38.8, 91.5]; novelty:

67.8 ± 71.9 [95% CI 40.5, 95.2]; structural: 91.6 ± 66.0 [95% CI 66.9, 116.2]).

Maze Training and Testing

The Kaplan–Meier estimates showed no effect of rearing treatment on the summed latency to eat during the maze arena training on the first day ($P = 0.74$) or the second day ($P = 0.21$; Table 3). All hens were faster to eat on the second day (Table 3). There were no differences between rearing treatments in the latency to eat when the arena was transformed into a maze ($P = 0.23$; Table 3), but hens took longer to eat compared with the previous sessions that day in just the open arena (Table 3). There was no effect of rearing treatment on the number of errors made during the maze test ($P = 0.78$) and overall, few errors were made (Table 3). Similarly, no treatment differences were found in the latency to leave the holding zone during the maze test ($P = 0.12$) and hens were quick to leave this zone (Table 3). There was no effect of rearing treatment in the latency to leave the holding zone when the novel object was present ($P = 0.74$), but hens took longer to leave the holding zone with the novel object present, with 31 hens never leaving the holding zone compared with zero hens not leaving before the novel object was present (Table 3). There was also no effect of rearing treatment on the latency to eat ($P = 0.90$; Table 3).

Personality Correlations

Across the 16 total correlations, 11 were significant for the control birds, six were significant for the novelty birds and seven were significant for the structural birds (Table 4). The birds showed correlations among tests across time indicative of consistent personality differences between individuals. Some correlations were not significant across all three rearing treatments, with three correlations showing significant results for the control and structural birds, but not the novelty birds, and one correlation showing a significant result for the control and novelty birds, but not the structural birds (Table 4).

The control hens showed a significant negative association between their latency to vocalize in the open field test and their mean daily range visits ($P = 0.008$) as well as a significant negative association between their latency to eat in the maze and their mean daily range visits ($P = 0.03$; Table 5). There were significant negative associations between the latency to leave the holding zone when the novel object was present during the maze test with both mean daily time outside ($P = 0.01$) and mean daily range visits ($P = 0.001$; Table 5). There were no significant relationships for the enriched hens (all $P \geq 0.34$; Table 5).

There were significant positive correlations in the mean daily time outside between the early and late ranging periods for the novelty and structural hens (both $P \leq 0.04$) but not for the control

Table 1

Mean \pm SD latency (s) for pullets from different rearing enrichment treatments (control, novelty, structural) to first step, to vocalize and to reach the final zone 4 and the mean \pm SD number of steps, vocalizations and escape attempts measured in a novel arena test

Behaviours	Rearing treatments			Test statistics
	Control	Novelty	Structural	
Latency to first step	99.5 \pm 129.0 (50.4, 148.6)	152 \pm 174.2 (84.5, 219.5)	124.8 \pm 137.0 (73.6, 176.0)	$\chi^2_2 = 1.72$, $P = 0.42$
Latency to vocalize	51.8 \pm 76.5 (22.7, 80.9)	97.4 \pm 157.1 (36.37, 158.3)	80.1 \pm 131.3 (31.07, 129.1)	$\chi^2_2 = 2.28$, $P = 0.32$
Latency to reach zone 4	445.5 \pm 220.4 (361.6, 529.3)	386.6 \pm 233.2 (296.1, 477.0)	405.6 \pm 236.7 (317.2, 494.0)	$\chi^2_2 = 0.17$, $P = 0.92$
No. of steps	89.4 \pm 89.3 (55.4, 123.3)	97.2 \pm 96.5 (59.8, 134.6)	78.9 \pm 70.7 (52.5, 105.3)	$F_{2,5,71} = 0.04$, $P = 0.96$
No. of vocalizations	53.6 \pm 43.1 (37.2, 70.1)	37.1 \pm 39.1 (22.0, 52.3)	62.9 \pm 48.5 (44.8, 81.0)	$F_{2,5,25} = 1.60$, $P = 0.29$
No. of escapes	0.97 \pm 1.1 (0.57, 1.37)	0.8 \pm 1.0 (0.43, 1.22)	1.0 \pm 1.3 (0.52, 1.48)	$\chi^2_2 = 0.42$, $P = 0.81$

The test statistics represent comparisons among rearing treatments. The 95% confidence intervals of the means are reported within parentheses.

Table 2
Mean \pm SD latency (s) for pullets from different rearing treatments (control, novelty, structural) to first step, to vocalize, to reach the feed zone and to eat the food in the two adaptation sessions of the novel arena with food

Behaviours	Adaptation session	Rearing treatments		
		Control	Novelty	Structural
Latency to first step	One	121.9 \pm 157.7 (62.0, 181.9)	150.8 \pm 166.3 (86.3, 215.2)	109.4 \pm 114.0 (66.9, 152.0)
	Two	58.0 \pm 82.2 (26.7, 89.3)	61.8 \pm 62.1 (37.7, 85.8)	44.6 \pm 78.2 (15.4, 73.8)
	Test statistics	$\chi^2_1 = 3.21$, $P = 0.07$	$\chi^2_1 = 6.70$, $P = 0.01$	$\chi^2_1 = 7.74$, $P = 0.005$
Latency to vocalize	One	145.9 \pm 227.6 (59.3, 232.5)	122.3 \pm 182.7 (51.5, 193.1)	81.6 \pm 153.9 (24.1, 139.1)
	Two	51.3 \pm 152.7 (–6.8, 109.4)	47.8 \pm 113.9 (3.6, 91.9)	37.8 \pm 108.8 (–2.8, 78.5)
	Test statistics	$\chi^2_1 = 2.80$, $P = 0.09$	$\chi^2_1 = 4.18$, $P = 0.04$	$\chi^2_1 = 3.30$, $P = 0.07$
Latency to reach feed zone	One	262.2 \pm 255.0 (165.2, 359.2)	270.4 \pm 231.8 (180.5, 360.3)	281.7 \pm 217.8 (200.3, 363.0)
	Two	165.1 \pm 203.6 (87.8, 242.5)	193.3 \pm 188.4 (120.3, 266.4)	158.5 \pm 194.6 (85.8, 231.1)
	Test statistics	$\chi^2_1 = 2.90$, $P = 0.09$	$\chi^2_1 = 3.11$, $P = 0.08$	$\chi^2_1 = 4.49$, $P = 0.03$
Latency to eat	One	495.8 \pm 181.2 (426.8, 564.7)	495.8 \pm 155.2 (435.6, 555.9)	489.8 \pm 168.8 (426.8, 552.9)
	Two	385.2 \pm 219.9 (301.6, 468.9)	360.9 \pm 236.2 (269.3, 452.5)	408.1 \pm 244.7 (316.7, 499.4)
	Test statistics	$Z = -1.66$, $P = 0.10$	$Z = -2.07$, $P = 0.04$	$Z = -1.14$, $P = 0.26$

Test statistics are for comparisons between the two adaptation sessions separately for each rearing treatment, with significant P values indicated in bold. The 95% confidence intervals of the means are reported within parentheses.

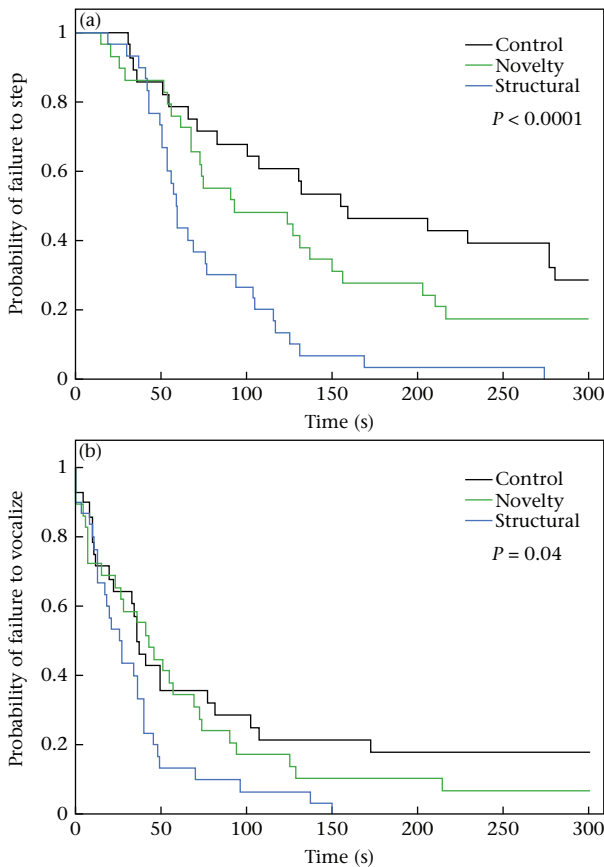


Figure 3. Latencies to exhibit behaviours in an open field test. The Kaplan–Meier curve for the time (s) to (a) first step and (b) first vocalize in the open field test for hens from three rearing enrichment treatments (control, novelty, structural). Significant P values are indicated in each plot.

hens ($P = 0.07$; Fig. 4). There were no significant correlations in the mean daily visits between the early and late ranging periods for any rearing treatment groups (all $P \geq 0.39$; Fig. 4).

DISCUSSION

The aim of this study was to assess the impacts of rearing enrichments on responses to different behavioural tests in free-range hens across pullet and adult stages including testing for stable personality differences and whether they predicted individual variation in early ranging behaviour. The two types of rearing enrichment reduced measures of fear and enhanced adaptation to new environments in pullets and young adult hens but inconsistently across varying test parameters. Within most test measures, the enriched birds did not differ significantly from the control birds. However, when test parameters were assessed across time, control birds showed a higher number of significant correlations than both types of enriched birds. This variation among all tested individuals and significant correlations among measures across time supports the presence of different personalities in laying hens. The control hens were the only birds to show significant relationships between some behavioural test measures and subsequent range use. These results suggest enriched hens developed a more plastic personality type that enabled greater change across time and adaptation to different environments with which they were presented. This type of response strategy may have fitness benefits for free-range birds or other domesticated or captive-reared animals that undergo drastic environmental change across their lifetime.

Contrary to expectations, there were few significant differences between the rearing treatments for individual test measures. Based on previous research, we predicted that enriched birds would show reduced fear, increased exploration, faster adaptation across time, quicker maze performance and greater boldness than the control birds. Enrichments in the form of varying novel objects and visual stimuli have previously resulted in reduced fear responses and increased exploration in young chicks as measured across five different behavioural tests (Jones & Waddington, 1992). Similarly, early stimulatory and physical enrichments have increased learning speed in a maze task in pullets tested prior to onset of laying (Campbell, Talk, et al., 2018). Rearing in a more complex environment has reduced fear responses in adult hens (Brantsæter et al., 2016), and exposure to structurally complex rearing environments has improved working memory and spatial detour task performance in young adults and chicks (Norman et al., 2019;

Table 3

Latency (s) for hens from each rearing treatment (control, novelty, structural) to eat during maze arena training (summed across five sessions on day 1, three sessions on day 2); latency for hens from each treatment to leave the holding zone (HZ) and to eat and the number of maze errors during the maze test; latency for hens from each treatment to leave the holding zone (HZ) and to eat during the maze test with a novel object present

Parameters	Rearing treatment			Test statistics
	Control	Novelty	Structural	
Maze arena training				
Summed latency to eat (day 1)	298.3 ± 224.0 (203.7, 392.9)	334.4 ± 210.1 (243.6, 425.3)	288.5 ± 176.8 (221.2, 355.7)	$\chi^2_2 = 0.60, P = 0.74$
Summed latency to eat (day 2)	56.6 ± 66.9 (28.36, 84.8)	37.0 ± 28.8 (24.5, 49.4)	58.2 ± 59.2 (35.7, 80.8)	$\chi^2_2 = 3.08, P = 0.21$
Maze test				
Latency to leave HZ	1.7 ± 1.8 (0.95, 2.5)	3.1 ± 4.9 (1.0, 5.2)	3.6 ± 4.1 (2.0, 5.2)	$\chi^2_2 = 4.19, P = 0.12$
Latency to eat	125.9 ± 113.1 (78.1, 174.6)	80.5 ± 92.8 (40.4, 120.6)	109.2 ± 95.7 (72.8, 145.6)	$\chi^2_2 = 2.91, P = 0.23$
No. of maze errors	0.7 ± 1.4 (0.13, 1.29)	0.4 ± 0.7 (0.04, 0.66)	0.7 ± 1.3 (0.18, 1.13)	$\chi^2_2 = 0.49, P = 0.78$
Maze test with novel object				
Latency to leave HZ	209.5 ± 120.1 (158.8, 260.2)	193.0 ± 111.0 (145.0, 241.1)	201.0 ± 101.7 (162.3, 239.6)	$\chi^2_2 = 0.72, P = 0.70$
Latency to eat	221.8 ± 116.8 (172.5, 271.1)	219.2 ± 107.7 (172.6, 265.8)	216.6 ± 96.3 (180.0, 253.3)	$\chi^2_2 = 0.29, P = 0.86$

Values are means ± SD. Test statistics are for comparisons between rearing treatments for each measure. The 95% confidence intervals of the means are reported within parentheses.

Tahamtani et al., 2015). Enriched-reared pullets did show faster adaptation across time, and the structural hens were quicker to first move in the open field test. The lack of consistent differences among treatments may indicate that some of the tests applied were either too provoking and many birds reacted with maximum fear, or did not adequately challenge the birds to detect differences, thus approaching floor and ceiling effects of individual variation. For

example, 47% of birds never made it to the final zone in the novel arena test, 60% of subjects did not eat in the first adaptation with food session and 70% of birds completed the maze training on day 2 in under 50 s with a range of latencies up to 280 s.

The differences in the rearing environments may have also not been contrasting enough to induce large differences in personality traits as measured by the specific tests in this study. Previous

Table 4

Spearman's correlations and *P* values for pairwise comparisons between behavioural parameters (latencies, in seconds) measured across different tests for birds from three rearing treatments (control, novelty, structural)

Behavioural parameters		Rearing treatment		
		Control	Novelty	Structural
Novel arena test as pullets				
Latency to step	Summed latency to eat adapt.	$r_s = 0.40$ (−0.004, 0.69), <i>N</i> = 24, <i>P</i> = 0.05	$r_s = 0.50$ (0.11, 0.76), <i>N</i> = 23, <i>P</i> = 0.02	$r_s = 0.21$ (−0.17, 0.54), <i>N</i> = 29, <i>P</i> = 0.28
Latency to step	Latency to eat day 1, maze arena	$r_s = 0.25$ (−0.17, 0.59), <i>N</i> = 24, <i>P</i> = 0.23	$r_s = 0.22$ (−0.21, 0.58), <i>N</i> = 23, <i>P</i> = 0.32	$r_s = 0.24$ (−0.14, 0.56), <i>N</i> = 29, <i>P</i> = 0.20
Latency to step	Latency to eat in maze	$r_s = 0.36$ (−0.05, 0.67), <i>N</i> = 24, <i>P</i> < 0.08	$r_s = 0.24$ (−0.19, 0.59), <i>N</i> = 23, <i>P</i> = 0.27	$r_s = 0.21$ (−0.17, 0.54), <i>N</i> = 29, <i>P</i> = 0.27
Latency to step	Latency to leave HZ NO	$r_s = 0.27$ (−0.15, 0.61), <i>N</i> = 24, <i>P</i> < 0.21	$r_s = 0.26$ (−0.17, 0.61), <i>N</i> = 23, <i>P</i> = 0.23	$r_s = -0.04$ (−0.4, 0.33), <i>N</i> = 29, <i>P</i> = 0.83
Summed latency to eat adapt.	Latency to eat, day 1 maze arena	$r_s = 0.68$ (0.38, 0.85), <i>N</i> = 24, <i>P</i> = 0.0003	$r_s = 0.43$ (0.02, 0.72), <i>N</i> = 23, <i>P</i> = 0.04	$r_s = 0.54$ (0.22, 0.76), <i>N</i> = 29, <i>P</i> = 0.002
Summed latency to eat adapt.	Summed latency to eat, training sessions 2–5	$r_s = 0.67$ (0.36, 0.85), <i>N</i> = 24, <i>P</i> = 0.0004	$r_s = 0.50$ (0.11, 0.76), <i>N</i> = 23, <i>P</i> = 0.02	$r_s = 0.73$ (0.50, 0.87), <i>N</i> = 29, <i>P</i> < 0.0001
Open field test as adults				
Latency to step	Latency to step NA	$r_s = 0.50$ (0.12, 0.75), <i>N</i> = 24, <i>P</i> < 0.01	$r_s = -0.01$ (−0.42, 0.40), <i>N</i> = 23, <i>P</i> = 0.96	$r_s = 0.21$ (−0.17, 0.54), <i>N</i> = 29, <i>P</i> = 0.27
	Latency to eat, day 1 maze arena	$r_s = 0.50$ (0.12, 0.75), <i>N</i> = 24, <i>P</i> < 0.01	$r_s = 0.62$ (0.28, 0.82), <i>N</i> = 23, <i>P</i> = 0.002	$r_s = -0.06$ (−0.42, 0.31), <i>N</i> = 29, <i>P</i> = 0.74
Latency to step	Latency to eat in maze	$r_s = 0.67$ (0.37, 0.85), <i>N</i> = 24, <i>P</i> < 0.0003	$r_s = 0.05$ (−0.37, 0.45), <i>N</i> = 23, <i>P</i> = 0.81	$r_s = 0.19$ (−0.19, 0.52), <i>N</i> = 29, <i>P</i> = 0.32
Latency to vocalize	Latency to vocalize NA	$r_s = 0.44$ (0.05, 0.72), <i>N</i> = 24, <i>P</i> < 0.03	$r_s = 0.19$ (−0.24, 0.56), <i>N</i> = 23, <i>P</i> = 0.40	$r_s = 0.58$ (0.27, 0.78), <i>N</i> = 29, <i>P</i> = 0.001
Latency to vocalize	Latency to step in OFT	$r_s = 0.61$ (0.28, 0.81), <i>N</i> = 24, <i>P</i> < 0.002	$r_s = 0.75$ (0.49, 0.89), <i>N</i> = 23, <i>P</i> < 0.0001	$r_s = 0.55$ (0.23, 0.76), <i>N</i> = 29, <i>P</i> = 0.002
Maze training and testing as adults				
Summed latency to eat, training sessions 2–5	Summed latency to eat, training sessions 6–8	$r_s = 0.75$ (0.50, 0.86), <i>N</i> = 24, <i>P</i> < 0.0001	$r_s = 0.82$ (0.62, 0.92), <i>N</i> = 23, <i>P</i> < 0.0001	$r_s = 0.67$ (0.40, 0.83), <i>N</i> = 29, <i>P</i> < 0.0001
Summed latency to eat, training sessions 2–5	Latency to eat in maze	$r_s = 0.59$ (0.25, 0.80), <i>N</i> = 24, <i>P</i> < 0.002	$r_s = -0.09$ (−0.48, 0.33), <i>N</i> = 23, <i>P</i> = 0.68	$r_s = 0.59$ (0.29, 0.79), <i>N</i> = 29, <i>P</i> = 0.0009
Latency to eat, day 1 maze arena	Latency to leave HZ NO	$r_s = 0.16$ (−0.26, 0.53), <i>N</i> = 24, <i>P</i> = 0.46	$r_s = -0.09$ (−0.48, 0.33), <i>N</i> = 23, <i>P</i> = 0.69	$r_s = 0.02$ (−0.35, 0.38), <i>N</i> = 29, <i>P</i> = 0.92
Summed latency to eat, training sessions 2–5	Latency to leave HZ NO	$r_s = 0.42$ (0.02, 0.70), <i>N</i> = 24, <i>P</i> < 0.04	$r_s = -0.25$ (−0.6, 0.18), <i>N</i> = 23, <i>P</i> = 0.25	$r_s = 0.08$ (−0.30, 0.43), <i>N</i> = 29, <i>P</i> = 0.69
Latency to eat in maze	Latency to leave HZ NO	$r_s = 0.76$ (0.52, 0.89), <i>N</i> = 24, <i>P</i> < 0.0001	$r_s = 0.23$ (−0.20, 0.59), <i>N</i> = 23, <i>P</i> = 0.28	$r_s = 0.43$ (0.08, 0.69), <i>N</i> = 29, <i>P</i> = 0.02

NA: novel arena test; OFT: open field test; HZ NO: holding zone with novel object present. Summed latency to eat is presented for the two adaptation (adapt.) sessions with food for pullets. Summed latency for adults to eat on day 1 during sessions 2–5 in the maze arena and on day 2 during sessions 6–8 in the maze arena. Significant *P* values are indicated in bold. The 95% confidence intervals reported in parentheses were calculated at <http://vassarstats.net/rho.html> (accessed 31 March 2021).

Table 5
Spearman's correlations and *P* values for pairwise comparisons between four latency (s) parameters measured across different tests and the mean daily time spent ranging and mean daily range visits during the early ranging period (27–31 weeks) for hens from each rearing enrichment treatment (control, novelty, structural)

Behavioural test	Range use	Rearing treatment		
		Control	Novelty	Structural
Latency to vocalize in OFT	Mean time outside	$r_s = -0.37$ (−0.67,0.04), <i>N</i> = 24, <i>P</i> = 0.08	$r_s = -0.09$ (−0.48,0.33), <i>N</i> = 23, <i>P</i> = 0.67	$r_s = -0.13$ (−0.47,0.25), <i>N</i> = 29, <i>P</i> = 0.52
	Mean visits	$r_s = -0.53$ (−0.77,−0.16), <i>N</i> = 24, <i>P</i> = 0.008	$r_s = 0.06$ (−0.36,0.46), <i>N</i> = 23, <i>P</i> = 0.77	$r_s = -0.10$ (−0.45,0.28), <i>N</i> = 29, <i>P</i> = 0.60
Summed latency to eat in training sessions 2–5	Mean time outside	$r_s = -0.15$ (−0.52,0.27), <i>N</i> = 24, <i>P</i> = 0.50	$r_s = 0.20$ (−0.23,0.57), <i>N</i> = 23, <i>P</i> = 0.37	$r_s = -0.04$ (−0.4,0.33), <i>N</i> = 29, <i>P</i> = 0.85
	Mean visits	$r_s = -0.20$ (−0.56,0.22), <i>N</i> = 24, <i>P</i> = 0.36	$r_s = 0.19$ (−0.24, 0.56), <i>N</i> = 23, <i>P</i> = 0.38	$r_s = -0.04$ (−0.4,0.33), <i>N</i> = 29, <i>P</i> = 0.85
Latency to eat in maze	Mean time outside	$r_s = -0.32$ (−0.64,0.10), <i>N</i> = 24, <i>P</i> = 0.13	$r_s = -0.08$ (−0.48,0.34), <i>N</i> = 23, <i>P</i> = 0.72	$r_s = -0.07$ (−0.43,0.30), <i>N</i> = 29, <i>P</i> = 0.74
	Mean visits	$r_s = -0.44$ (−0.72,−0.05), <i>N</i> = 24, <i>P</i> = 0.03	$r_s = -0.21$ (−0.57,0.22), <i>N</i> = 23, <i>P</i> < 0.34	$r_s = -0.07$ (−0.43,0.30), <i>N</i> = 29, <i>P</i> = 0.71
Latency to leave HZ NO	Mean time outside	$r_s = -0.50$ (−0.75,−0.12), <i>N</i> = 24, <i>P</i> = 0.01	$r_s = -0.06$ (−0.46,0.36), <i>N</i> = 23, <i>P</i> = 0.77	$r_s = -0.13$ (−0.47,0.25), <i>N</i> = 29, <i>P</i> = 0.51
	Mean visits	$r_s = -0.66$ (−0.84,−0.35), <i>N</i> = 24, <i>P</i> < 0.001	$r_s = 0.11$ (−0.32,0.50), <i>N</i> = 23, <i>P</i> = 0.62	$r_s = -0.13$ (−0.47,0.25), <i>N</i> = 29, <i>P</i> = 0.51

OFT: open field test; HZ NO: holding zone with the novel object present. Summed latency is presented for training sessions 2–5 in the maze arena training on day 1. Significant *P* values are indicated in bold. The 95% confidence intervals reported in parentheses were calculated at <http://vassarstats.net/rho.html> (accessed 31 March 2021).

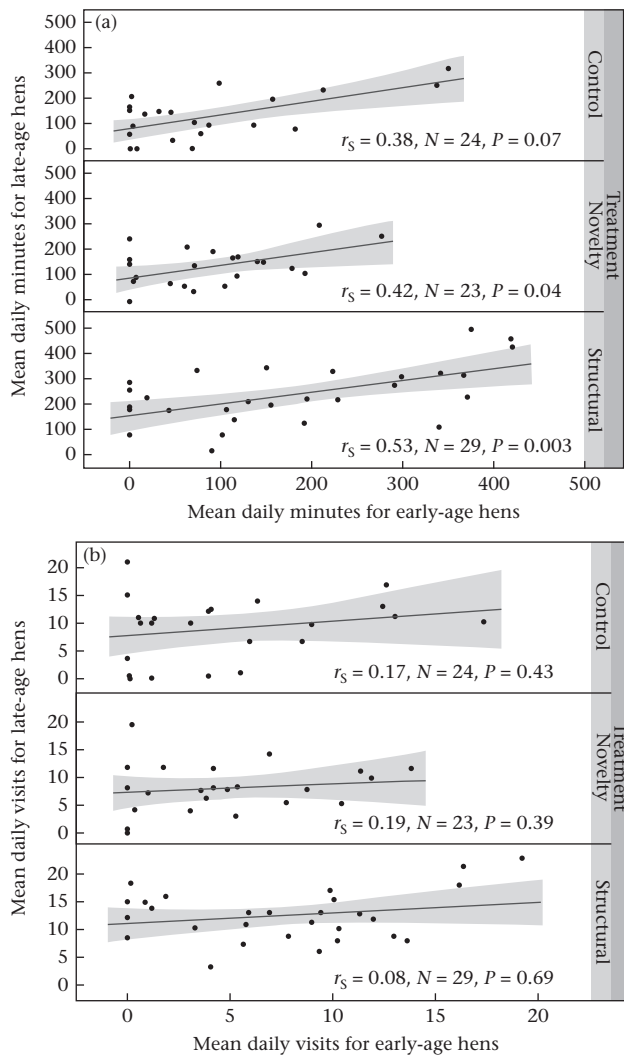


Figure 4. Early and late ranging behaviour of hens from different rearing enrichment treatments. The relationship of the (a) mean daily minutes spent outside and (b) mean daily visits outside between early (weeks 27–31) and late (weeks 62–64) ages for hens from three rearing enrichment treatments (control, novelty, structural). The Spearman's correlation and *P* values between ages are included for each treatment group.

studies showing fear and cognitive impacts of rearing environments have contrasted cage rearing with aviary rearing (Brantsæter et al., 2016; Tahamtani et al., 2015), which is likely a greater contrast than the current study, although Norman et al. (2019) compared presence or absence of perching structures in floor litter pens, which is similar to the treatments applied here. There were rearing treatment differences in behaviour and welfare measures throughout the flock cycle as demonstrated in the larger trial (Bari, Cohen-Barnhouse et al., 2020; Bari, Downing et al., 2020; Campbell et al., 2020). Across all birds, the structural hens showed longer daily total ranging time, the novelty hens had the longest individual visit times to the range (Campbell et al., 2020) and the control hens showed the worst plumage condition across time (Bari, Cohen-Barnhouse et al., 2020; Bari, Downing et al., 2020). Few differences were detected across rearing treatments in behavioural tests of tonic immobility, open field and emergence on a different subset of adult hens at 62–63 weeks of age (Bari, Allen, Mesken, Cohen-Barnhouse, & Campbell, 2021).

While there were few differences among rearing treatments within individual test parameters, there were significant correlations in measures made across time with fewer correlations in the enriched birds. These results confirm the presence of interindividual personality differences in laying hens and suggest a more plastic personality ontogeny in enriched birds. The greater number of correlations across time in the control birds was further confirmed by these birds being the only group that showed predictive associations between test measures and subsequent range use. The significant correlations suggest that range use in this sample of control hens was related to their response to fear-inducing novel environmental surroundings, as well as spatial navigation. Across all treatment groups, there were significant correlations between the latencies to eat during the adaptation sessions as pullets and the latencies to eat during the maze training measured approximately 10 weeks later. However, adult hens were substantially quicker to eat compared with the pullets, further confirming how values of specific measures may change with sexual maturity, but among-individual personality variation is retained (Groothuis & Trillmich, 2011; Stamps & Groothuis, 2010). Specifically, in these brown strains of laying hens, there is substantial contrast in the behaviour of pullets compared with adults (Campbell, Talk, et al., 2018). Pullets will avoid personnel, but once sexually mature, hens will actively approach people; this change in

human-related fear also translates to responses in behavioural tests that may elicit fear in the birds. In contrast, there were no relationships for any birds in the latency to first step in the novel arena as pullets and the first latency to eat in the maze training arena, latency to eat in the maze test and the latency to leave the holding zone when the novel object was present in the maze test. This suggests that these test measures were assessing contrasting aspects of the bird's personality, that the trait is context specific or that the first time in the novel arena as pullets was overstimulating for all birds, resulting in a maximum fear response regardless of potential individual personality variation. The exact same behavioural tests were not applied in the pullets and adults due to the observed differences in fear and difficulty in training pullets to walk to the end of the arena when they were unwilling to eat in the test arena. It is uncertain whether there would have been greater consistency in adult birds across age versus between pullet and adult stages in the same individuals.

There was also no relationship for any birds in the latency to first eat in the maze training arena and the latency to leave the holding zone in the presence of the novel object. While both conditions exposed hens to novelty, the novel environment versus a novel object may be measuring different underlying traits of exploration versus fear, respectively, and thus may not be expected to correlate, as also shown in other avian species (Fox, Ladage, Roth, & Pravosudov, 2009). The majority of other correlations among test variables across time showed varied significance among the three treatment groups, where the significant correlations in the control birds sometimes matched those in the structural hens and the novelty hens, or did not match either. In other species, environmental enrichment improves behavioural and cognitive flexibility on specific tasks (reviewed in Gelfo, 2019), but the impacts of enrichment on ontogenetic plasticity are less well studied. Across species, specific developmental experiences or environments will affect the degree of consistency in personality across time. For example, the positive or negative experience of watching a winner or loser during fights in rainbow trout, *Oncorhynchus mykiss*, altered the degree of shyness or boldness across time relative to controls that had no specific experiences (Frost, Winrow-Giffen, Ashley, & Sneddon, 2007), and exposure to predation resulted in a correlation between boldness and aggressiveness in threespine sticklebacks, *Gasterosteus aculeatus*, not previously present (Bell & Sih, 2007).

Provision of enrichments may have stimulated adaptation to change, although the precise mechanism for this is uncertain. It is highly probable that the enrichments resulted in neurological changes (Ohline & Abraham, 2019) and the enriched birds may have had higher synaptic plasticity, which in turn affected the plasticity of their personality development. Evidence across multiple species confirms the increasing neural plasticity with provision of environmental enrichment (e.g. Kempermann, 2019; Novkovic, Mittmann, & Manahan-Vaughan, 2014; Salvanes et al., 2013), but the precise mechanisms and brain regions involved remain to be determined. It is possible that the different enrichment strategies resulted in varying degrees of brain lateralization in the birds, which increased their hemispheric flexibility and influenced their responses to different testing environments (Rogers, 2012; Rogers & Kaplan, 2019), a hypothesis that requires further research. In addition to the effects of the rearing enrichments, ranging itself may have also affected behaviour and neural development. While time spent ranging showed similarities between early and later range use, the daily visits to the range did not match, indicating that range use itself may play a role in modifying a bird's behaviour. These results are similar to those of the overall flock study, where the lowest relationships in range use patterns were seen across the first and last measurement time points in the flock

cycle compared with successive time points (Campbell et al., 2020). Recent evidence indicates that more time spent outside was positively associated with greater hippocampal cell proliferation (Armstrong et al., 2020).

Regardless of the exact mechanism involved, a more plastic developmental trajectory may enable an animal to better respond and adapt to environmental change. This is particularly pertinent to free-range birds given the drastic changes between indoor rearing and outdoor access as adults. This is coupled with frequent changes in their surrounding outdoor environment (e.g. changing weather, predation risk) or variable management strategies associated with managing birds with outdoor access and the need to minimize exposure to extreme environmental conditions (e.g. thunderstorms, heat waves). In the larger study, the structural birds showed the longest ranging time (Campbell et al., 2020) and the novelty birds showed greater use of the indoor tiered nestbox (Bari, Cohen-Barnhouse, & Campbell, 2020), suggesting better adaptation to the range and indoor layer pen, respectively. Across age, the control hens had the worst plumage condition as they got older, which indicates presence of detrimental feather-pecking behaviour within the group (Bari, Downing, et al., 2020). This may be a fitness consequence of a poorer adaptation strategy in these birds for that type of housing environment. Similar types of results have been seen in captive-reared animals destined for wild release, where those that were exposed to enriched complex environments had greater success upon release (Atlantic salmon, *Salmo salar*: Mes et al., 2019). Furthermore, more behaviourally flexible animals are also proposed to be more successful invasive species (Sol & Lefebvre, 2003).

Overall, enriched rearing environments had some impacts on reducing fear and increasing adaptation in laying hen pullets and adults with greater consistency in test parameter correlations across time in the control birds. Range use was only able to be predicted in the control hens with measures that suggest ranging is related to fear-inducing novel environmental surroundings and spatial navigation. The plasticity in the enriched hens may allow better adaptation to the free-range environment, resulting in improved fitness. Further work should confirm the impacts of enrichment on ontogenetic plasticity in laying hens and other species that undergo drastic environmental change across their lifetime.

Data Statement

The data that support this study can be found at: <https://data.csiro.au/collections/collection/Clcsi:49883v1>.

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