**PROJECT 1: ESTIMATING HERITABILITY**

For this practical you will take advantage of an experiment that was designed to measure the heritability of human seeking behaviour in a specially bred dog population. This dog population was a pedigree of beagles that were specifically bred under controlled conditions with a known pedigree. This pedigree then allows us to assess the heritability of this behaviour. Your task will be to take this data and then finish the experiment! You need to analyse the data in any way you see fit and assess the heritability of the behaviour you have measured. Once you have your results you need to write them up in a similar manner to a scientific journal (you can be a little more verbose however – there are no page charges!). Finally discuss your results, paying special attention to the ramifications and potential problems with estimating heritability in this manner.

**BACKGROUND**

In this project, we performed a behavioural test, targeting the communicative skills of dogs, on a large, well defined population of laboratory beagles, bred and housed under highly standardized conditions. The test utilized the so-called ‘unsolvable problem’ paradigm (Topal *et al.* 1997). In this, dogs are allowed to explore an unsolvable food-search problem and the propensity of the dog to seek human con- tact and cooperation is measured. The aims were to evaluate within-breed variation and heritability in human-directed con- tact seeking behaviour.

**METHODS**

***Housing and handling***

Beagles, bred and kept under standardized conditions, at a research dog kennel were used in this study. In total there were approximately 10 caretakers, both male and female, rotating their tasks and care for the dogs and facilities. One member of the staff would therefore not necessarily always work in the same stables. Whelping and nursing was carried out in a separate nursery unit, where the dogs were kept until eight weeks of age. After weaning, they were housed in groups of 2–4 same sexed (usually not from the same litter) individuals in pens, composed of both an indoor (2 – 3 × 3 – 4 m) and an outdoor area (3 – 4 × 6 m) with gravel and sand mixed flooring, elevated platforms and chewing toys which they had constant access to.

The dogs were fed in the morning, once a day at the same time and usually in the outside pens and they had a constant *ad libitum* supply of water. Puppies were handled and socialized according to a predetermined schedule, specifying activities on a weekly basis from 4 until 13 weeks of age. Until the dogs were 11 months of age they were weighed, had their claws cut and were trained to walk on a leash on a monthly basis.

The staff at the kennel performed a simple sociability test at 5 and 11 months of age, and at 49 weeks a final test was carried out, in order to judge the suitability of the dogs for participation in different medical research projects. In addition, handling of dogs was scheduled for nail-cutting, and they were moved to a larger outdoor play pen for a couple of hours every second week or when being rearranged to other pens. When being moved to play pens the keepers let them walk on a leash. Dogs were handled outside of the schedule whenever they needed veterinary attention. Apart from this, dogs were not handled.

***Animals tested***

All tests, as well as handling prior to and during testing, was carried out by the same female experimenter (first author) and were stan- dardized and performed according to what is described here. In total, 498 beagles were all tested once each. We first carried out an initial feeding test, investigating whether dogs were qualified to perform the actual behaviour test (see below for details of this). Out of the 498 dogs, 437 qualified for the behaviour test (196 males and 241 females, median age 1.3years). The majority of dogs tested were young (388 individuals ranging from 8 months to 2.4 years of age), but breeding animals were also tested (49 individuals ranging from 2 to 6 years of age).

Most of the beagles had at least one parent for which the pedigree could be traced back on the kennel for 6 – 10 generations. However, 14 individuals had been imported from other research kennels and were not related to the rest of the dogs. These individuals were excluded from genetic analysis.

**Procedure**

The same female experimenter handled the dogs during the testing (from the point of collecting them in their home pens until they were put back again), and also carried out the behaviour tests. The experimenter captured dogs in a random order, one at a time in their home pens, and when placed back, the next dog was taken from another pen. A martingale collar with a leash was used when walking the dogs from their home pen to the procedure room. Dogs that would not walk on the leash were instead picked up and carried to the procedure room. While the dogs were on the leash just outside their home pen, approximately five attempts were performed to achieve eye contact. The experimenter did this by first kneeling down (hunching down with one or two knees on the ground) and talking to the dog in a calm and encouraging manner (talking in normal to low volume with slightly higher pitch than normal, with the intention of being positive and inviting). If the dog would not look at or seek eye contact with the experimenter within approximately 20 seconds, the leash was slowly shortened until the experimenter could softly pat the dog and make eye contact. After this, it was usually enough to talk encouraging to the dog for it to again seek eye contact. Otherwise, the previous procedure was repeated once more, so a maximum of two times, which was enough for all dogs to seek eye contact with the experimenter. If the dog did not immediately seek eye contact when the experimenter was kneeling down, the procedure took about one minute. Otherwise, the duration of this eye contact establishing procedure was slightly shorter. As it was repeated 5 times, it took about 5 min in total for each dog. The collar was removed upon entering the test room and the dog was allowed to move around freely for approximately 2 min. The test room was approximately 3.5 X 4 m, empty from furniture, and windows were covered.

The ID of the dog was checked and noted by reading the ear tattoo. This was then written on a small whiteboard presented in front of the video camera (Canon Legria HF M52) as the video recording was started for each dog (see below). Continuous recording took place from this point forward, until the dog either failed the initial feeding test or until the end of the entire test.

***The initial feeding test and the Feeding Score***

In order to test how willing the dogs were to eat the treats, they were presented with a treat (quarter pieces of normal sized FROLIC®) on a single plate, similar to those used in the test setup later but without a cover (Fig. 1). After placing the single plate containing a treat on the floor, the test leader was standing passively in the same position. If the dog ate a treat, another was placed on the plate, without picking it up, until the dog had eaten a total of three treats. The initial feeding test had a maximum duration of 2 min. However, some individuals would continue to explore the procedure room by sniffing the floor and walls instead of having their visual attention directed towards the plate. Therefore, if the dog did not show interest in (visual and physical orientation towards the object) the plate within approximately 30 seconds, the experimenter tried to redirect its attention towards the treat by placing it directly on the floor within 20 cm of the plate. If the dog was not visually orientated towards the treat while it was placed on the floor, the test leader would talk to the dog and indicate the location of the treat by pointing at it with her right hand until she was sure the dog had seen the treat, and then she went back to standing passively. Most dogs would eat the treat when placed on the floor. The next treat was again initially placed on the plate, but moved to the floor next to the plate if the dog did not eat it within 30 seconds. If the dog had not eaten all treats after approximately two minutes from when the plate was first put down, the dog did not qualify for the unsolvable task. On the other hand, if the dog ate all the treats it had qualified, and the plate was replaced with the unsolvable task. During testing, a stopwatch was used to keep track of the time recording, however, later these video sections were analysed using The Observer XT 10 software. Feeding was scored based upon the latency until eating the first treat (Feeding Score) for each individual on a scale from 1–3 where 1 was late feeding, 2 was medium feeding and 3 was early feeding (Table 1).

***The unsolvable task***

The device for the unsolvable task consisted of three plates (Fig. 1) on a solid foundation, each covered with transparent Plexiglas lids with six 0.5 mm odour ports. The test setup was placed on the floor approximately 15 – 20 cm from the wall. Two of the three plastic covers could be easily pushed to the side giving access to the treatA score from 1–3 explaining the time it took for the dog to eat all treats presented on the single plate during the pre-test. Dogs that did not eat all three treats within 2 minutes were excluded from the study and did not perform the problem-solving test.

underneath, while the third lid (the middle one) could not be opened. The experimenter sat on a stool approximately 1.5 m from the test setup facing towards it. During three minutes, the dog was allowed to freely move around the room and manipulate the unsolvable task. If the dog had not succeeded to reach any of the treats after 60 seconds, the experimenter opened both plastic covers halfway and sat down again. The total duration of the test was 3 minutes. After each test, the floor and the entire test equipment was cleaned and prepared for the next dog.

***Data analysis, ethogram and scoring***

Videos were analysed and behaviours were scored using The Observer XT 10 software. For every subject, ID, sex, date of birth, date and time of testing were noted. The ethogram used for the recordings is shown in Table 2. For each behaviour, frequency, latency and duration were recorded and the latencies for the dog to solve the first and the second solvable tasks were noted. Additionally, a ‘Transition Index’ was calculated by summing up the total number of direct transitions, which the dog made between the experimenter and the test setup. Direct transitions were those where the dogs’ head did not leave the zone, within its own body length, of the test setup before entering the zone of the experimenter, and vice versa. From each video recording, the dogs’ Feeding Score (Table 1), as previously mentioned, and Body Posture Score (Table 3) were scored onascalefrom1to5where1islowbodyandtailpostureand5is high body and tail posture.

***Pedigree***

Dogs tested were all from an outbred population of research beagles. Astra Zeneca provided the pedigree used for breeding purposes. By tracing the ancestry of the 437 tested dogs, we ended up with a total of 643 individuals in the pedigree used for heritability calcula- tions. For one of the tested dogs, ancestry was unknown, so it was excluded from further analysis. The tested individuals belonged to 160 different litters.

***Statistical analysis of behaviour***

All statistical analyses, except for heritability calculations, were per- formed in IBM SPSS Statistics 22.

First, a Principal Component Analysis was carried out on all the behavioural variables. Sampling adequacy: Bartlett’s sphericity test *𝜒* 2 = 7899.66, df = 210, p *<* 0.001; KMO: 0.855. Eigenvalue *>* 1 was the criterion used to determine the four principal components used for further analysis and no factor rotation was used. (The original cor- relation matrix can be found in the supplementary material, Table S1)

Data was checked for normality, both visually and with the Kolmogorov–Smirnov test, and, if necessary, transformed (log10 (x + 1)). Then, effects of sex and age as well as their interaction were investigated using Univariate General Linear Models in SPSS Statis- tics 22. Effects of age, sex and their interactions were investigated for each behavioural variable and for the scores on each of the four Principal Components. In the model, sex was set as a fixed factor and age as a covariate. If the interaction was not significant, it was removed from the model and only the main effects of sex and age were used in the analysis. Age ranged from 0.7 to 6.2 years with an average age of 1.5 years and a median age of 1.3 years.

