Social Cognitive Evolution in Captive Foxes Is a Correlated By-Product of Experimental Domestication

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Supplemental Experimental Procedures

Details of Selection on Experimental and Control Foxes

The experimental and control populations were founded from commercial stock in 1959 and have subsequently been maintained in identical conditions. However, the experimental population of foxes was selected for breeding based on standardized behavioral measures since 1959 [S1, S2]. Starting at 1 month old, each experimental subject was tested for its reaction to an experimenter. Once a month during infancy, an experimenter would attempt to stroke and handle each kit while offering it food. In addition, it was observed whether kits preferred to associate with other kits or the experimenter. Once the kits reached sexual maturity, at 7–8 months old, each subject was tested for a final time and was assigned an overall tameness score based on a strict set of behavioral criteria. First, experimenters independently rated each subject's tendency to approach them while they were standing in front of their home pen. Second, experimenters independently rated each subject's tendency to bite at them if they tried to touch it. Within the experimentally selected population, only those individuals who were least fearful and aggressive toward a human (evidenced by their approach behavior and attempts to initiate human contact) were selected for breeding. Less than 20% of each successive generation in the experimental population were allowed to breed each year [S1, S2].

On the basis of this single selection criterion, behavioral, physiological, and morphological changes that were either not found in control foxes or were expressed at a significantly higher frequency in the experimental population were observed in the experimental fox population. First, the individuals in the experimental population were observed from 1 month of age onward to show little fear or aggression toward humans in that they were more "eager to establish human contact, whimpering to attract attention and sniffing and licking the experimenters like dogs" (page 163 in [S2]). Subsequent experiments have demonstrated that selection for tameness retarded the development of fear responses to novelty and enhanced exploratory behavior in unfamiliar situations [S3]. Second, physiological changes were observed after several generations of selection. The first change detected was attenuated activity of the pituitary-adrenal axis. The common pool of circulating glucocorticoids, their in vitro production, the basal level of the adrenocorticotrophic hormone (ACTH), and the adrenal response to stress were all reduced in the experimental foxes in relation to controls [S4]. Subsequently, the activities of the serotonin, noradrenaline, and dopamine transmitter systems in specific brain regions that are implicated in the regulation of emotional-defensive responses have also been found to be altered in the experimental foxes [S5, S6]. Finally, and perhaps most surprising, the domesticated foxes manifested a number of morphological changes that are commonly found in a number of other domesticated species. A higher frequency of domesticated foxes had floppy ears, short or curly tails, depigmentation of hair, extended reproductive seasons, and even changes in the size and shape of the crania and dentition [S1, S2, S7].

The results of the fox farm experiment demonstrate that selection against aggressive and fearful behavior not only leads to changes in behavior, but also results in a number of other correlated or unselected modifications in the mammalian phenotype (similar effects of selection against aggression and fear have been shown in mink and rats [S2]). The presence of such correlated by-products raises the possibility that selection for tame behavior may also have correlated effects on the cognitive abilities of domesticated canids.

Experiment One: Do Experimental Foxes Use Human Communicative Cues to Find Food?

Subjects

Eleven experimentally domesticated foxes (six females and five males) and eleven dog puppies (four females and seven males) were compared. All of the foxes tested in this and subsequent studies were born and raised at the experimental farm of the Institute for Cytology and Genetics in Siberia, Russia. Subjects were selected to be tested during an initial screening in which the boldest individuals between 2 and 4 months old (the youngest available at the time) were chosen to participate. All of the foxes tested were born between April and May, 2003. Until they were 6–8 weeks old, the subjects lived with their mother and littermates. Subjects were then housed with their littermates until they were approximately 12 weeks old. After this period, they were singly housed in a wire mesh pen ($90 \times 75 \times 70$ cm). While living in their pen, subjects could interact at anytime with neighboring foxes in adjacent pens. In addition, each subject was fed a fresh mixture of meat, fish, corn, vegetables, and vitamin supplements in a metal bowl twice daily. Before participating in the current investigation, the subjects were only handled for management purposes (approximately twice a month). Therefore, subjects had minimal interactions with humans, typically only seeing someone briefly when their food and water bowls were refilled each day (this is the same for the control foxes used in later studies). On testing days, subjects were given their morning meal immediately after completing the test session. The 11 domestic dog puppies had previously been tested [S8]. As with the foxes, all of the puppies in this sample were 2–4 months of age when they were tested.

Procedure

Each subject was brought into the testing room (3×4 m) for three to four play sessions in which they were free to interact with the experimenter for 45 min to ensure that the subjects were accustomed to the experimenter and testing room (the experimenter sat on the floor and waited for the subject to initiate interactions). After each subject readily took food from the experimenter's hand and out of the bowls to be used in the test, it was introduced to the general testing procedure [S8]. Each subject was taken from its home pen to the familiar testing room, where two hemispherical bowls (20 cm diameter and 8 cm tall) had been placed 1.7 meters apart in the middle of the room. A line that was equidistant between the two bowls was drawn in the middle of the room. Then, a subject was shown that food could potentially be found in both hiding locations. One experimenter (E1) placed a small piece of food (i.e., fresh cheese) in one of the two bowls while the subject watched while being held by a second experiment (E2) 1.5 m away from each bowl. Once the food was hidden in one of the bowls, the subject was released by E2 to retrieve the food (E2's eyes remained closed throughout a trial so that they did not know where the food was baited). If the subject first touched the bowl where the food was hidden, it received the reward, whereas if it touched the empty bowl first, it was shown the location of the food but did not receive the reward. Subjects only entered the testing phase after successfully finding the hidden food in four out of five consecutive trials during this introduction phase.

The experimental session was similar to the introduction and occurred on a separate day. E1 placed the two bowls 1.7 m apart while E2 held the subject approximately 1.5 m away from the bowls. After showing the subject a piece of food, E1 then sham-baited both bowls, only leaving food in one of the two bowls. Therefore, the subjects knew food was hidden, but did not know in which bowl. Once the food was hidden, E1 attracted the attention of the subject (i.e., by snapping), and then, while lying on the ground (to ensure

that the subject would see E), indicated the location of the food by pointing and gazing toward it. The experimenter extended her arm and index finger in the direction of the hidden food while also gazing at the baited bowl.

Once E was pointing and gazing at the correct bowl, E2 released the subject to retrieve the food while E1 continued giving the cue as the subject approached one of the bowls. If the subject chose the correct container, it was rewarded. If it chose the wrong container, it was shown where the food was hidden but did not receive the treat. In addition, after the completion of all testing, the experimental foxes were given a control test. This condition was identical to that just described, with the exception that the experimenter did not provide a cue to the food's location after it was hidden. Instead, the experimenter only stared directly at the subject until it made its choice.

Subjects received 18 trials in each condition in separate sessions. Food was never hidden in one of the bowls for more than two trials in a row, and the food placement was counterbalanced within and across subjects. Subjects' choices were scored live during testing. Subjects were tested in no more than one session per day, and all trials were videotaped from behind E1. Within groups, performance was assessed by comparing overall number of correct choices to chance with a one-sample t test. Between group performances were compared with Welch independent sample t tests. Because of our a priori and directional predictions (see Introduction), all tests were one-tailed unless otherwise noted (this is true throughout all four experiments).

Experiment Two: Do Experimental or Control Foxes Use Human Gestures More?

Subjects

Seventeen experimentally domesticated (nine females and eight males) and seventeen control-line foxes participated (nine females and eight males). None of these subjects participated in experiment one. All of the fox kits were born in April, 2003 and were between 3 and 4 months old when tested.

Apparatus

Subjects were tested in their home pens, which were rectangular and made of wire mesh (90 $\times$ 75 $\times$ 70 cm). During testing, the neighboring foxes, living in pens on either side of the subject's pen, were removed, and opaque barriers were placed inside these pens, making it impossible for the subject to see any other conspecific during testing. A rectangular board (80 $\times$ 30 cm) with a line down the middle was placed in the center of the floor of the subject's home cage; subjects quickly developed a preference for sitting upon it. A table was built that could be placed against the front of a fox's home cage. Attached to the table top (70 $\times$ 50 cm) was a board (70 $\times$ 20 cm) that could slide across the table top 40 cm. Attached to either end of the board were two wooden cubes (10 cm^3), each of which had a long, thin piece of flexible metal (15 $\times$ 1 cm) that extended 10 cm in front of the sliding board (the metal was cut from a retractable ruler or tape measure). When pressed down and then released, the flexible metal (i.e., the toy) would return to its original position and make a small noise in the process. There was 60 cm between the two metal toys. Wooden sticks (75 cm $\times$ 2 cm) were placed on the pen's mesh front to aid the experimenter with coding. One stick was placed vertically on the center of the mesh front, whereas the remaining two sticks were placed 20 cm from the center.

Procedure

Introduction: On their first day, all subjects participated in an introduction. In this introduction, the experimenter conducted three manipulations, including (1) approaching the subject's pen until she was standing an arm's length away and could touch the wire mesh with one hand, (2) placing the apparatus against the front of the subject's pen before leaving, or (3) not placing herself or the apparatus near the subject's pen. Each manipulation lasted 3 min. Half of the subjects within each group were presented with the experimenter first, and half with the apparatus first. Subsequently, the remaining two manipulations were completed and also counterbalanced within each group.

Test: Each subject was tested at least 1 day after its introduction. The apparatus was again placed in front of the subject's home cage while the experimenter sat behind it. Once a subject was seated in the center of the pen, the experimenter gazed at, gestured toward, and touched one of the two metal toys with her contralateral arm so that it moved and made a small noise.

Once the experimenter gave the cue, the sliding board was pushed forward so that the metal toys were within reach of the subject (10 cm inside its cage). The subjects were then observed for 30 s. If they did not touch one of the toys in this time, the sliding board was moved so that both toys were again removed from the subjects' reach. If the subject did touch one of the toys, the experimenter recorded which of the toys the subject touched first. Subjects were allowed to play with the toys regardless of whether they touched the same toy as the experimenter. The toys were then removed after 5–10 s of play. Each subject participated in a single 18-trial session. The same metal toy was never manipulated for more than two trials in a row, and the order of the toy manipulation by the experimenter in each session was counterbalanced within and between subjects.

Scoring and Analysis

Within the introduction, the subject's time to first approach was coded in the manipulations involving either the experimenter or apparatus. A first approach was scored from videotape as the time between the start of a trial (when the human or apparatus first touched the pen) and the time at which a subject first touched the experimenter or apparatus with its mouth or paw. Within the test session, the experimenter coded live which of the two toys the subject first touched with its mouth or paw. All introduction and test trials were videotaped so that both the subject and the experimenter were visible.

Experiment Three: Are Experimental Foxes More Skilled than Controls in All Human-Led Tasks?

The method used in the current experiment was identical to experiment two, except for the following changes: First, only a subsample of subjects were tested from experiment one (experimental: three females, six males; control: five females, four males), on the basis of their level of participation in experiment two. In each group, the nine subjects who participated at the highest level in experiment two were chosen for testing in experiment three. Once chosen, an analysis comparing the overall level of participation between this subset of experimental and control foxes showed that this subset from each group also did not differ in their level of participation in experiment two ($t[13.14] = 1.24$, p = NS, Welch independent sample t test). Yet even when compared on their first five trials, the nine experimental foxes touched the toy the experimenter manipulated significantly more than the nine control foxes ($t[12.57] = 3.11$, $p < 0.005$, Welch independent t test; Figure 2). Therefore, in experiment three each subject participated in a five-trial session to test its preference for touching a toy an object had manipulated. Second, two additional pieces of equipment were added to the apparatus: (1) an opaque occluder (80 $\times$ 30 cm) and (2) a feather attached to a wooden stick (50 cm). The opaque occluder could be placed on the table top, thus shielding the movements of the experimenter's arms and hands from the subject's view. The feather was then used to manipulate one of the two metal toys and made a small noise from behind the occluder in each trial. Therefore, although the subject could see the human experimenter's face, shoulders, and legs, it could not see that the experimenter moved the feather from behind the occluder with the aid of the stick. Third, there was no introduction session. Fourth, subjects only received five trials with the object cue.

Experiment Four: Are Experimental Foxes More Skillful with Human Communicative Cues than Controls?

The same method was used in this experiment as was used in experiment one, with a few exceptions. First, the performance of six 2–3-month-old control fox kits (the youngest available; four females and two males) was compared to the previous performance from experiment one of six age-matched experimental fox kits (four females and two males). Second, unlike the experimental foxes, these six control foxes were brought into the testing room every

day for several weeks so that they could play and interact with the experimenters for 1–2 hr a day (these play periods were carried out as experiments one to three were being conducted). Third, the control foxes were only tested with the point-and-gaze cue.

Supplemental References

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