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Public opinion and the scientific literature alike reflect a widespread assumption that there are differences in behavior between dog breeds. Direct empirical behavioral assessments of such differences, however, are rare and have produced mixed results. One area where breed differences are often assumed is olfaction, where German Shepherds, hounds, and Labradors are commonly used for odor-detection work, whereas toy breeds and brachycephalic dogs, such as Pugs, are not. Choice of breed for scent detection work, however, may be driven more by historical choices than data. In this article we directly assessed the ability of German Shepherds, Pugs, and Greyhounds to acquire a simple olfactory discrimination, and their ability to maintain performance when the target odorant was diluted. Our results show that contrary to expectations, Pugs significantly outperformed the German Shepherds in acquiring the odor discrimination and maintaining performance when the odorant concentration was decreased. Nine of 10 Greyhounds did not complete acquisition training because they failed a motivation criterion. These results indicate that Pugs outperformed German Shepherds in the dimensions of olfaction assessed. Greyhounds showed a general failure to participate. Overall, our results highlight the importance of direct behavioral measurement of assumed behavioral breed differences.

Keywords: olfaction, canine, domestic dogs, scent detection, breed differences

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Exploring and comparing the sensory capacities of different species is important in understanding an organism's *Umwelt*. Identifying the range of environmental stimuli an animal is sensitive to is critical in understanding their behavior and perception of the world. Despite the increasing scientific study of dogs in recent years (Feuerbacher & Wynne, 2011) and dogs' wide use as odor detectors, very little research has explored canine olfactory perception, which is widely assumed to be a large component of a dog's experience (Horowitz & Hecht, 2014). In particular, even less work has looked at how physiological differences across breeds might influence olfactory perception.

Dog breeds are a potentially useful model for comparative work in olfaction. Not only are dogs particularly sensitive to odorants (Krestel, Passe, Smith, & Jonsson, 1984; Passe & Walker, 1985; Walker et al., 2006), but dog breeds manifest a relatively extreme range of phenotypic diversity among closely related individuals

that has only recently evolved, probably in the last several hundred years (Vonholdt et al., 2010). There are over 170 unique breeds recognized by the American Kennel Club alone (www.akc.org/breeds/complete_breed_list.cfm), with many other organizations recognizing other breeds. Each of these breeds is largely a genetically isolated population and is described by the organizations that register these dogs as phenotypically distinct (morphologically and behaviorally) from all other breeds. The morphological differences between breeds are readily recognizable; however, how differences such as brachycephaly translate into olfactory perceptual differences is less clear.

Direct comparative analyses of olfactory behavior are critical to understanding the relationship between olfactory physiology and olfactory experience. One example of the importance of comparative behavioral assessment comes from comparisons between primate olfaction and that of rodents and dogs. Assumptions based on the number of functional olfactory receptor genes, have led to suggestions that primates possess reduced olfactory sensitivity and are "microsomatic" in comparison with "macrosomatic" species like the mouse or dog (e.g., Rouquier, Blancher, & Giorgi, 2000). Recent behavioral work, however, has called these interpretations into question (Laska & Seibt, 2002; Laska, Seibt, & Weber, 2000; Laska, Wieser, & Hernandez Salazar, 2005; Laska, Wieser, Bautista, & Hernandez Salazar, 2004). Primates are surprisingly sensitive to odorants showing sensitivity to some odorants comparable with that of rodents and dogs (Laska et al., 2000). These findings highlight the importance of direct behavioral assessment when comparing the sensory experiences of different species.

In terms of dog breeds, German Shepherds, Terriers, and Labradors are commonly seen performing odor detection work for police (e.g., Jezierski et al., 2014) and military forces (e.g., Sinn, Gosling, & Hilliard, 2010). In contrast, toy and brachycephalic breeds are rarely used for such tasks. This disproportionate use of a few breeds in scent-detection work has led some researchers to assume that there are important behavioral differences in the domain of olfactorily guided behavior. For example, Robin et al. (2009) assessed the diversity of olfactory receptor polymorphisms across breeds and found that some polymorphisms were breed specific. Robin et al. took this evidence as an explanation for why Labrador retrievers are more commonly deployed as sniffer dogs than Pekingese or Greyhounds. Similarly, Roberts et al. (2010) noted that brachycephalic breeds show a repositioning of the olfactory lobe, and offered this as a potential explanation for why such dogs are not used for scent-detection work. Critically, however, no empirical research has directly tested whether behavioral differences do indeed exist between these breeds with respect to olfaction.

To date, only a few studies have investigated breed differences in scent detection capacities. Some studies have found indirect evidence that breeds may differ in olfactory capacity by looking at differences in olfactory receptor (OR) genes. Tacher et al. (2005) sequenced 16 OR genes in 95 dogs from 20 breeds and found high levels of allelic polymorphism, with some polymorphisms being breed specific. Robin et al. (2009) extended this research confirming high levels of allelic polymorphisms. Different breeds also showed different levels of polymorphism for different genes, and interestingly, 16 of the 109 sequenced ORs had alleles leading to pseudogenes. Overall, these studies showed clear differences between breeds in terms of olfactory receptor polymorphisms. More important, however, it is unclear what behavioral effect, if any, these OR polymorphisms may have in creating breed differences in olfaction. Given the complexity of the olfactory receptor code, it is unknown how these breed differences in OR genes translate to differences in olfactory perception or olfactorily guided behavior (for a review, see Quignon, Rimbault, Robin, & Galibert, 2012).

Some studies have sought to characterize *behavioral* breed differences in scent-detection tasks. Rooney and Bradshaw (2004) compared search dog handlers' responses on a survey as a function of the breed of their search dog and found that overall satisfaction with the dog did not depend on breed. There were differences, however, in other behavioral dimensions, such as motivation to obtain food. Using a similar procedure Adamkiewicz et al. (2013) surveyed dog trainers and handlers of drug- and explosives-detection dogs for both German Shepherds and Labrador retrievers. Although there were inconsistencies between handler reports and trainer reports, a few differences between Labradors and German Shepherds were noted. These differences, however, were on dimensions such as "friendliness to people," "stamina," "ability to concentrate," and others, which at best, are only indirectly relevant to the task of detecting odors. The trainers and handlers failed to report any breed differences in willingness to sniff objects. Trainers, but not handlers, scored Labradors trained for drug detection as having a better sense of smell than similarly trained German Shepherds. This difference in trainer scores, however, was not seen for dogs trained to find explosives. Overall, these observations do not provide strong evidence of breed differences in olfactory capabilities.

Recently, Jezierski et al. (2014) assessed the performance of 161 dogs from four breeds trained by the Polish police to detect narcotics (Labrador Retrievers, German Shepherds, Terriers, and English Cocker Spaniels). The dogs were all selected and fully trained by the police before entering the study. Jezierski et al. found that German Shepherds showed a significantly superior performance in indicating narcotics (86% correct indications) than Labradors (79% correct indications), and Terriers (67% correct indications).

Thus, few empirical studies support the notion of breed differences in olfactory detection performance, although such differences have been widely assumed. More generally, only a handful of empirical studies have been conducted to directly assess any behavioral breed differences (for a review, see Mehrkam & Wynne, 2014). Some assumptions, however, regarding behavioral breed differences do appear to survive empirical testing, such as broader-headed dogs being able to pull more weight than more narrow-headed dogs (Helton, 2011). The cephalic shape also appears to influence people's assumptions regarding a dog's trainability (Helton, 2009), and perceived trainability ratings might be more closely related to physical features of the dog rather than their cognitive ability (Helton, 2010).

The aim of the present study is to directly compare dog breeds' olfactory discrimination performance. To test this, we assessed the number of trials needed to reach criterion on an odor discrimination (acquisition) and subsequent performance when the target odor concentration is varied. For this study we selected Pugs, German Shepherds, and Greyhounds for comparison. We selected German Shepherds because they are commonly used for scent detection work. We selected Pugs, because they are a toy breed and brachycephalic, making them a likely candidate for a poor olfactory performance because of potential rotation of the olfactory lobe or crowded ethmoturbinate bones as a consequence of brachycephaly (Roberts et al., 2010). We selected Greyhounds because they are considered sight hounds and not scent hounds (Parker, 2012). Thus, according to the traditional classification of dogs, they should be relatively unsuited to olfactory learning. We hypothesized that German Shepherds would excel at learning the task whereas Pugs and Greyhounds would not. We hypothesized that additional differences would be observed when the odorant is diluted. We expected German Shepherds to maintain a stable performance, but the brachycephalic breed (Pugs) would show a rapid decrease in performance indicating an inability to detect the diluted odorant.

Method

Acquisition

Dogs of three different breeds were trained to alert to a novel target odorant in a discrete-trials, two-choice odor discrimination task that has previously been utilized by Hall, Smith, and Wynne (2013) and Hall, Smith, and Wynne (2014). In this procedure, dogs are trained to dig in a bucket of pine shavings containing the target odorant, and refrain from digging in an identical bin of pine shavings without the target odorant. The aim of the present experiment was to compare the rate of acquisition of this olfactory task across breeds.

Subjects

German Shepherds, Pugs, and Greyhounds living as pets in people's homes were recruited for the present experiment. Dogs were recruited via mass emailed solicitations to a participant pool, flyers at dog parks, emails through breed club listservs, and by word of mouth. The inclusion criteria required dogs to be a healthy purebred member of their breed and naïve to previous odor detection training. To meet the breed and health criteria, owners needed to confirm the breed of their dog and that it showed no signs of current illness. Breed was confirmed by the owner indicating they had acquired the dog from a breeder or breed specific rescue organization, and an experimenter visually confirmed the dog met breed color, size, and morphological breed expectations (experimenter N.H or K.G.). In total, 10 German Shepherds, 11 Pugs, and 10 Greyhounds were recruited and met inclusion criteria. One Pug and nine Greyhounds failed to meet motivation criterion during testing by failing to take a visible and freely available treat on two presentations of the treat for two consecutive sessions. These dogs were not tested further (details described below), leaving a final sample size of 10 German Shepherds (four males, six females; mean age = 4.2 years, age range: 1–8 years), 10 Pugs (four males, six females; mean age = 6.3 years, age range: 3–9 years), and one Greyhound (one male, 5 years).

Materials and Procedure

The target odor (S+) was prepared by placing 1 ml of anise extract (McCormick, Sparks, MD) on a cotton round (100% cotton). The cotton round was then buried 2 cm deep into a Sterilite bin (30 cm × 36 cm × 15 cm) filled 8 cm deep with PetsPick pine shavings (American Wood Fibers, Columbia, MD). The nontarget odor bin was prepared identically, except that 1 ml of mineral oil (S−) was placed on the cotton round instead of the target odorant (anise extract). Mineral oil was used as the nontarget odorant because it was used in the subsequent dilution phase.

Alert training. Dogs were first trained to root in the bin of pine shavings as an indicator response in eight discrete trials. At the start of each trial, an assistant held the dog at least 2 m back from the testing area. The Experimenter (E) then placed a treat into a bin of anise-scented pine shavings, placing it on top of the pine shavings so that it was readily visible. The bin was then placed down on the ground 2 m away from the dog and the assistant released the dog. The dog was allowed to eat the treat. Once the dog ate the treat, E said, "good dog" and gave the dog a treat by hand. This trial was repeated once more. Next, three identical trials were conducted, except that the treat was now buried in the pine, making the dog dig for the treat. Once the dog began to dig, E said, "good dog" and delivered a treat by hand. Last, another three trials were conducted in which no food was placed in the bin, but once the dog began to dig, E said, "good dog," and delivered a treat by hand.

Discrimination training. After completing alert training, dogs moved onto discrimination trials. At the start of each discrimination trial, E simultaneously placed one bin containing the target odor and one bin containing the nontarget (mineral oil) on the floor, 0.5 m apart, and equidistant from the dog that was held at least 2 m back by the assistant. The bins used during discrimination trials were separate from the bin used for alert training to prevent food contamination. After placing both bins down, E

stepped at least 1 m back, and assumed a neutral position not looking at the dog or either bin. The assistant then released the dog and observed it until it made a response. The assistant/observer was always blind to which bin contained the target odorant. When the assistant/observer saw the dog make a response to one of the bins, she called out "choice," to inform E that the dog had made a response, which prompted E to look at the dog and determine whether the response was correct. If the dog made a correct response, E delivered a treat. If the dog made an incorrect response, the bins were picked up until the next trial. If the dog did not respond to either bin within 30 s, E picked up the buckets and represented the trial. If the dog did not respond again within 30 s, a "no response" was recorded and scored as incorrect.

Controls. The present procedure is designed to prevent observer bias by having an assistant blind to which bin is correct score all the responses and to reduce unintentional cuing by having the E stand in a neutral posture away from the bins. To test the reliability of the observer's scoring, a second independent observer scored a subset of trials (235 trials) from video. The second observer agreed with the first observer, on average, for 94% of the trials (Cohen's κ, K = .87).

We also included two additional controls to test for unintentional cuing. Throughout all testing, a subset of trials conducted were control trials. For these trials, E prepared two bins using a procedure identical to that used to prepare the discrimination bins, except that neither contained the target odor and both contained the S−. One bin, however, was a priori designated as the target bin before preparation of the bins. During control trials, E conducted the trial as though it was a discrimination trial and reinforced responding to the a priori designated "correct" bin. If E was providing unintentional cues via handling, posture, or by any other means, dogs would perform above chance on control trials. If dogs, however, were only following the target odor, we would expect their performance to drop to chance, or they would fail to respond as the target odor is missing.

In addition to control trials, we also conducted a subset of acquisition and dilution trials double-blind. For double-blind trials, a third person arranged the bins and placed them into E's hands without E knowing which bin was the target. The third person then left the testing area and E conducted the trial. The assistant, as always, was also blind to the target bin, but still called choice after the dog responded. After the assistant called choice, the third person immediately entered the testing area (within seconds) and revealed which bin was the target. E then provided the appropriate consequence to the dog. We hypothesized that if E was unintentionally cuing the dogs, performance on double-blind trials would be significantly lower than performance on the nonblind trial immediately proceeding that trial.

Sessions. Dogs were given one session per day for a total of four acquisition sessions, which were scheduled according to owner availability. Each session started with eight alert-training trials that were initially used to train the digging response, but were subsequently used as "warm-up" trials. Dogs were then given 30 discrimination trial and 6 control trials. The order of discrimination trials and control trials for each phase is shown in Figure 1. For acquisition, control trials were presented after every five discrimination trials (see Figure 1). The location of the target bin was pseudorandomly determined so that no more than two trials in a row occurred with reinforcement to the same side. Six double-
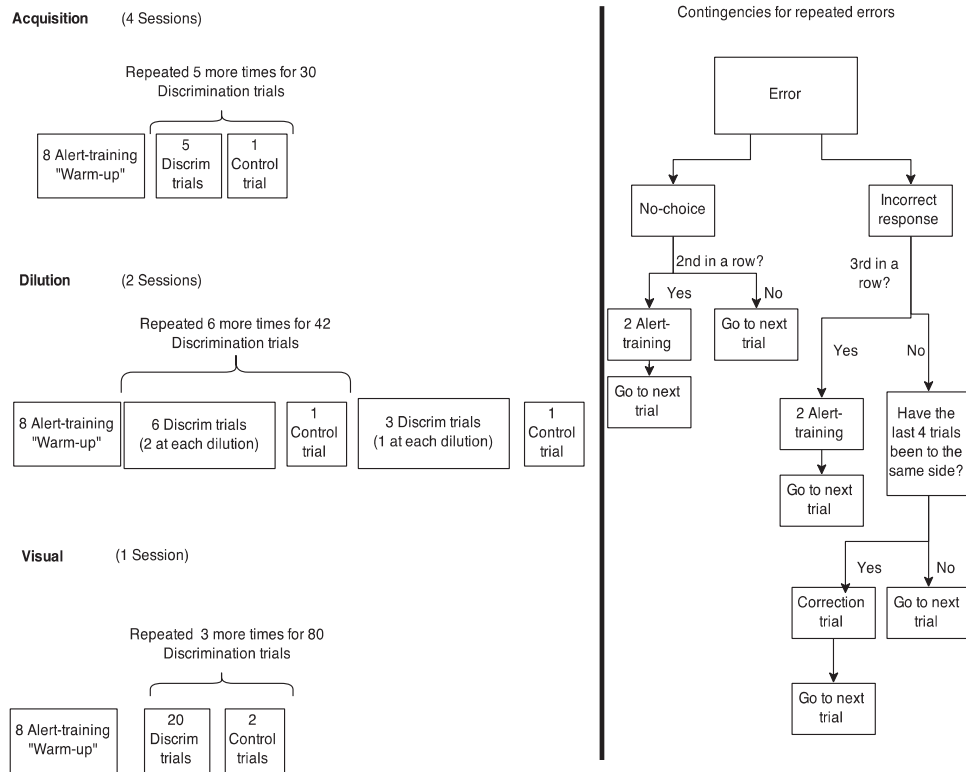
Figure 1. Experimental schematic. Top left shows the trial order for acquisition, which started with alert training followed by discrimination ("discrim") training. Left middle shows the trial sequence for the dilution phase and left bottom shows the trial sequence for the visual discrimination. The right shows the contingencies for additional alert-training trials or correction trials after making an error.

blind trials were conducted on two separate sessions of either acquisition or the dilution trials (dilution trials are described below) for each dog; except that no double-blind sessions were conducted for one German Shepherd nor the Greyhounds as they largely failed to complete the testing sessions. Additional trials were run during sessions with other German Shepherds so that a total of 120 double-blind trials were run with German Shepherds and 120 trials were run with Pugs. Double-blind trials were distributed across both acquisition and dilution sessions.

To facilitate training, if dogs made repeated errors to the same side, repeated incorrect responses, or simply failed to respond, additional alert-training trials or correction trials were conducted. Figure 1 highlights the contingencies under which these trials were conducted. If, during training the dog failed to respond for two trials in a row or made three incorrect responses in succession, two alert training trials in which the food was placed on top of the pine shavings were conducted. This was done to test motivation to participate. If the dog consumed the food for both trials, discrimination testing resumed with the next scheduled trial. If the dog failed to take the food when freely available within 2 min, the dog met the motivation criterion for stopping and testing was suspended for the day. If a dog made an error and had selected the same side for four successive trials, a correction trial was conducted to remove the side bias (see Figure 1). E conducted a correction by repeating the same trial, except that before the dog could approach the incorrect side, E picked up the incorrect bucket

forcing the dog to approach the alternative side. Digging in the alternative bin was reinforced.

One German shepherd failed the motivation criterion during the first session after completing 12 trials. Eight of 10 Greyhounds met this contingency for 2 days in a row and were dropped from the study. One further Greyhound was dropped after failing to complete any trials during the first testing session. This dog was no longer available for a subsequent motivation test. Thus, nine Greyhounds were dropped because of motivation. One Pug also met the motivation contingency. For this Pug, however, during the first session, the Pug fell into the bucket, flipping the bucket and briefly becoming trapped. After this event, the Pug refused to approach the bucket for free food for several days after, and was subsequently dropped from the study.

Dilution

All dogs that completed acquisition training (10 German Shepherds, 10 Pugs, and one Greyhound) were then tested on a set of odorant concentration trials in two sessions. The aim of these sessions was to rapidly assess the dogs' performance when the concentration of the target odorant was systematically reduced. To do this, dogs were given a mixture of trials in which the target odorant was either at the same strength as the initial training odorant, or an approximate 10% or 1% dilution.

Materials and Procedure

The full strength target odor (100%) was prepared identically as in acquisition trials (i.e., 1 ml of anise extract on a cotton round). Two emulsions of the target odor that approximated a 10% and 1% dilution were prepared using mineral oil. Mineral oil was used for its availability and convenience, but also because it allowed us to assess performance on a complex discrimination (i.e., mineral oil vs. mineral oil + target odor). The 10% odorant was prepared by mixing 1 ml of anise extract with 9 ml mineral oil. The solution was shaken vigorously until a complete emulsion formed. E then placed 1 ml of this emulsion onto a cotton round and buried it in a clean bin of pine shavings. To prepare the 1% dilution, E placed 1 ml of the 10% emulsion into 9 ml of mineral oil. The emulsion was then vigorously shaken and 1 ml was removed, placed on a cotton round, and buried in a clean bin of pine shavings. Thus, three S+ bins were prepared: one bin for the 100% odorant, one for the emulsion that approximated a 10% odorant dilution, and one for the emulsion approximating a 1% odorant dilution.

Discrimination trials. Trials were conducted with the same procedure as acquisition trials, except that the appropriate S+ stimulus for each trial was utilized. Dogs received a total of two training sessions, each consisting of alert training with the full dilution as "warm-up" trials, 45 discrimination trials (15 trials for each dilution) and eight control trials. The order of presentation of the 100%, 10%, and 1% trials was pseudorandomly assigned into blocks of trials so that no more than two trials of one dilution occurred without presentation of at least one of the other two dilutions (see Figure 1). Eight control trials were conducted in each session identically to the control trials during acquisition. Double-blind trials were conducted as described in the acquisition methods. Contingencies for correction trials, giving additional alert-training trials, and exclusion from the study were the same as in acquisition.

Visual

Eight of the Pugs, seven of the German Shepherds, and five of the Greyhounds that were recruited for the olfactory discrimination were available for an additional visual discrimination-training task. The visual discrimination was conducted to assess whether breed differences in the olfactory discrimination could be because of general learning or motivational differences rather than reflecting specific differences related to odor-discrimination. For this task, dogs were trained to discriminate between a tall cup and a short cup. The dogs from the two olfactory tasks were trained under the same settings and conditions as the olfactory discrimination.

Materials and Procedure

Dogs were trained in one session to discriminate between an inverted "short" cup with a height of 4 cm, from an inverted "tall" cup that was 14 cm tall (both cups had a diameter 6.4 cm). The cups were generic Styrofoam cups that were cut to the appropriate sizes and placed open-side down. The S+ cup (tall or short) was approximately counterbalanced across dogs.

At the start of the training session, each dog was presented with four response-training trials. For these trials, E placed the S+ cup with a treat on top it, prompting the dog to approach the cup and take the treat. These trials were similar to the odor-discrimination alert-training trials, and served to train the initial response and insure the subject was motivated. Once the dog took the treat from the cup, the experimenter delivered an additional treat by hand. This was repeated three more times to rapidly train a touching response to the cup.

Identically to the start of each trial for the odor discrimination procedure, E placed both the S+ and S− objects 0.5 m apart equidistant to the dog, which was held by an assistant at least 2 m away. E then stepped back at least 1 m, assumed a neutral position, and the assistant released the dog. A response was made once the dog physically touched a cup. If the dog touched the appropriate S+ cup, E said, "good dog," and gave the dog a treat. If the dog touched the S− first, both cups were picked up, and no treat was delivered. Contingencies for correction trials, scoring a "no response," being given additional response training, or being removed from the study were identical to the olfactory discrimination contingencies. Three of the five Greyhounds failed to complete the session: One completed the first block of 20 trials only, and two did not complete any trials.

Controls. Control trials were similar to the control trials used for the olfactory task. For visual control trials, two identical cups that met the criterion for the S− cup (short cup or tall cup) were used. One cup was a priori designated to E as the correct cup. E then conducted a normal trial. If the dog touched the a priori designated correct cup first, E delivered a treat. Otherwise, both cups were picked up. The purpose of these trials was to detect unintentional cuing by E, or other unintended cues. If dogs were only following the visual stimulus, we expected dogs to perform at chance levels or fail to respond. No double-blind trials were conducted for the visual discrimination, because we could not blind the assistant and the experimenter to whether a cup was tall or short without also removing their ability to see the dog.

Session. All dogs were trained on the visual discrimination during one session. The session comprised four blocks of 20 discrimination trials and two control trials each. Thus, the total session contained 80 discrimination trials and eight control trials. The position of the S+ was counterbalanced across trials and was pseudorandomly determined so that the S+ was not on the same side for more than two trials in a row.

Statistical Analysis

Data were analyzed and plotted using R (R Core Team, 2013). Confidence intervals (CIs) for plots were created using the package *ggplots2* (Wickham, 2009). To assess the effect of breed differences on proportion correct, we used linear mixed-effects models using the *lme4* package (Bates, Maechler, Bolker, & Walker, 2013) and the *lmerTest* package to compute p values (Kuznetsova, Brockhoff, & Christensen, 2014). An initial regression was fit with age, sex, breed, and session number as predictors of proportion correct. Nonsignificant predictors were removed during model selection by comparing models with and without a predictor term of interest using a likelihood ratio test (LRT) from the *pbkrtest* package (Halekoh & Hojsgaard, 2013).

Results

Acquisition

Both Pugs and German Shepherds showed increasing performance across sessions indicating that both breeds readily acquired the task. Greyhounds, however, largely failed to participate with only one dog completing acquisition. Therefore, we excluded Greyhounds from statistical analyses. Figure 2 (Acquisition) shows the mean performance and 95% CI of the Pugs ($n = 10$) and the German Shepherds ($n = 10$). Figure 2 (Acquisition) shows that Pugs consistently out-performed German Shepherds across all sessions, and by Session 4, the 95% CIs show no overlap. To assess statistical differences in performance, linear mixed-effects models were fit to the data. Model selection indicated that sex and age were unrelated to performance during acquisition (sex: $LRT = 1.58$, $p = .26$; age: $LRT = 3.49$, $p = .10$). The final model indicated that Pugs significantly outperformed German Shepherds ($t_{18} = 3.27$, $p < .01$; see Supplementary Table 1 for regression parameters). Pugs overall had a 0.16 greater proportion correct score than the German Shepherds (95% CI [0.06, 0.25]). There was also a significant increase in performance across sessions indicating that both breeds learned the task across sessions ($t_{58.95} = 7.80$, $p < .001$), with proportion correct increasing on average by .09 each session (95% CI [0.07, 0.11]).

When comparing breed performance, it is also informative to compare the numbers of individuals of a breed that were high performers. Figure 3 (Acquisition) shows the numbers of Pugs and German Shepherds that met an 83% correct criterion in each session (i.e., 25 out of 30 correct, binomial test, $p < .001$). Across sessions, more dogs of each breed met the individual criterion. By Session 4, nine of 10 Pugs were meeting criterion, whereas only three German Shepherds met this criterion. This indicates that uniformly as a breed, the Pugs were reaching a high accuracy (90% of the Pugs). Only a minority of German Shepherds met this criterion (30%) within four sessions.

Nine of the 10 Greyhounds failed the motivation criterion. Only one Greyhound completed acquisition, but several participated for several trials before failing the motivation criterion. Supplementary Figure 1 shows the performance of the Greyhounds and the performance of the one Greyhound that completed acquisition. This Greyhound, however, failed the motivation criterion during the subsequent dilution phase.

Dilution

Pugs also outperformed the German Shepherds on the dilution series. Figure 2 (Dilution) shows the mean performance of the Pugs and German Shepherds averaged across the two dilution sessions for the initial training odorant concentration (100% anise extract), and the two diluted odorants. The Pugs outperformed the German Shepherds on the initial training concentration. On the dilutions, Pugs showed no decline on the 10-fold dilution, whereas the German Shepherds did (Figure 2: Dilution). On the 100-fold dilution, Pugs still outperformed German Shepherds. The Pugs' mean performance even on the 100-fold dilution was greater than the German Shepherds mean performance on the 10-fold dilution.

To test for statistical differences in performance, a linear mixed-model was fit to the average performance for the Pugs and German Shepherds across each dilution with age and sex included as covariates (see Supplementary Table 2 for model specification). We subsequently reduced the model by removing the nonsignificant terms. There was no significant effect of sex ($LRT = .034$, $p > .05$), age ($LRT = 1.90$, $p > .05$), or interaction between the breeds and the dilutions ($LRT = 2.95$, $p > .05$). There was, however, a main effect of breed ($t_{18} = 4.30$, $p < .001$), in which Pugs showed an overall 0.18 greater proportion correct than the German Shepherds (95% CI [0.08, 0.21]). There was also a significant decrease of about 0.18 in proportion correct (95% CI [0.13, 0.22]) as the target odor was diluted down to 1%, $t = 7.38$, $p < .001$.
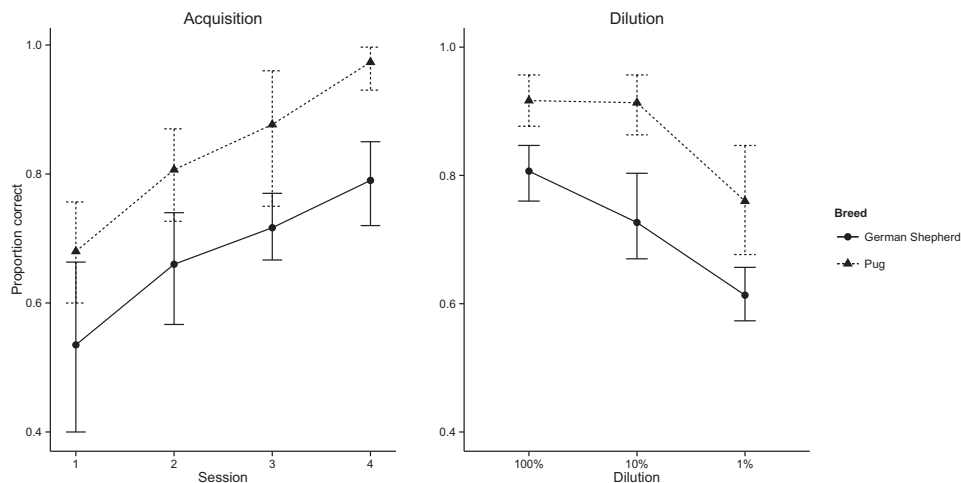

Figure 2. Mean accuracy during acquisition and dilutions. Lines show mean proportion correct for each breed across sessions for acquisition and the dilution series. Error bars show the 95% confidence interval computed using a bootstrapping procedure.
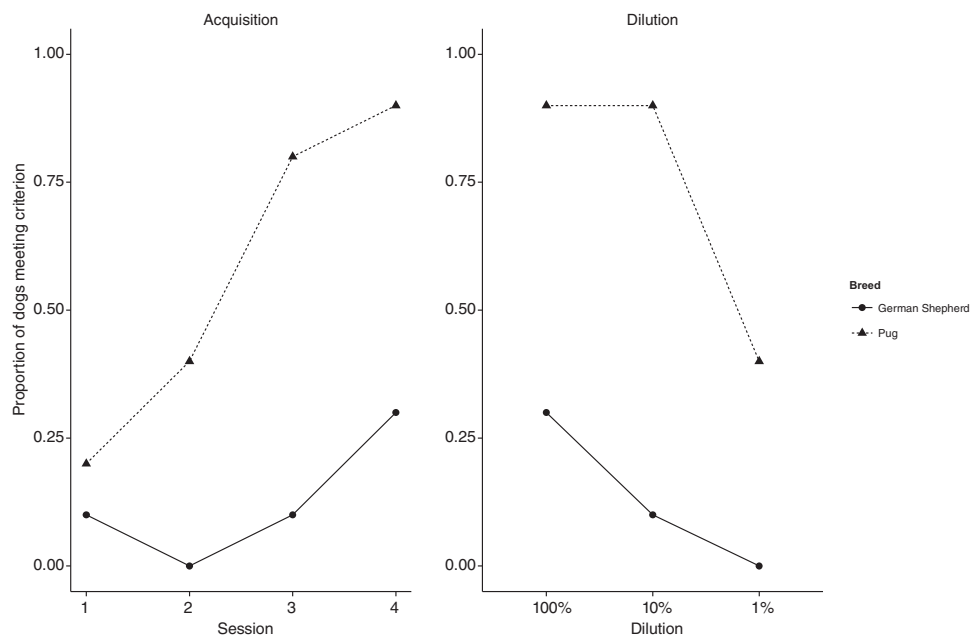
Figure 3. Proportion of dogs meeting individual criterion for success. Lines show the proportion of dogs in each breed group that met an 83% correct criterion for acquisition and the dilutions.

As in acquisition, it is also interesting here to compare the numbers of individuals in each breed meeting a high performance criterion of 85% correct (at least 25 out of 30 across both session, binomial test, $p < .001$). Figure 3 (Dilution) shows that nine of the nine Pugs that met criterion for the training odor also met criterion for the 10-fold dilution whereas only one of the three German Shepherds that met criterion for the concentrated odorant met the criterion for the 10-fold dilution. The authors found it interesting that there were four Pugs that continued to meet the 85% correct criterion on the 100-fold dilution whereas no German Shepherds met this criterion. Thus, not only did Pugs learn the olfactory discrimination faster, they were able to maintain performance with lower odorant concentration than the German Shepherds.

Controls

The difference in performance between Pugs and German Shepherds does not appear to be explained by the Pugs utilizing unintentional olfactory, visual, or experimenter-delivered cues. Performance on control trials never exceeded 50% for either breed during the acquisition or dilution sessions, indicating they were not utilizing unintentional odor or visual cues. In addition, a total of 240 trials distributed across the acquisition and dilution trials (120 trials with nine German Shepherds and 120 trials with 10 Pugs) were conducted double-blind. Overall mean percent correct on double-blind trials was 78% correct ($SD = 17.8\%$) whereas mean percent correct on single blind trials was 81% ($SD = 18.1\%$). Thus, percent accuracy on double-blind and single blind trials did not differ (paired t test $t_{18} = 1.03$, $p > .05$), indicating that the dogs were not utilizing unintentional experimenter cues.

Of interest to the authors, across the acquisition and dilution trials, we did detect a breed difference on control trials. As Pugs'

experience with the task increased, the likelihood the Pugs would respond during control trials decreased, even though food was available by chance 50% of the time. Instead, the Pugs would sniff both control buckets and refrain from responding to either. In contrast, German Shepherds readily responded during control trials. We fit a mixed model with for the number of "no responses" during control trials as a function of session number and breed. There was a significant breed and session interaction ($t_{98} = 6.42$, $p < .001$). Initially, Pugs and German Shepherds were equally likely to respond during control trials; however, as the Pugs learned the task, they became less likely to respond during control trials although there was a reinforcement contingency for responding randomly. German Shepherds did not. Thus, not only did Pugs show better performance during regular discrimination trials, they also showed a spontaneous refusal to respond during controls when neither bin held the target odor. This suggests that the Pugs' performance was strongly guided by the presence of the target odor.

Although we insured all dogs were motivated to participate by excluding dogs that failed a motivation test, it is possible that the differences we observed between Pugs and German Shepherds may not relate to olfaction per se, but may be a product of breed differences in food motivation, trainability, or general learning processes. To assess whether these differences were olfaction specific we compared performance of the Pugs, German Shepherds, and Greyhounds on a simple visual discrimination. If Pugs outperform German Shepherds on the visual task, this suggests that our results with olfactory stimuli may reflect breed differences in motivation or trainability. A lack of difference or if the German Shepherds outperform the Pugs, will suggest that the differences observed on the olfactory tests are olfaction specific.

Visual

Both Pugs and German Shepherds showed steady acquisition of the visual discrimination in the allocated trials. Unlike the olfactory discrimination, however, the Pugs and German Shepherds acquired the task at a similar rate (see Figure 4). For the Pugs and German Shepherds, the CIs for each block of trials overlap and there is no clear separation between the two breeds. We fit a linear mixed-model to performance on the visual discrimination as a function of breed (German Shepherds and Pugs) and block of session as fixed effects. There was a significant effect of block of trials as both German Shepherds and Pugs showed an increasing trend ($t_{43.95} = 6.22$, $p < .001$), but there was no difference between the two breeds ($t_{13} = -0.44$, $p > .05$).

Acquisition of the visual discrimination overall did not appear to be influenced by unintentional cuing. Mean percent correct on control trials was 51% correct, whereas chance performance was 50% (German Shepherds: 53% correct, Pugs: 48% correct). Thus, the dogs appeared to be attending to the visual stimulus and not any unintentional cues.

Figure 4 also shows the proportion of subjects meeting an individual criterion (German Shepherds, $n = 7$; Pugs, $n = 8$). We lowered the criterion for individual "success" to 75% (15 correct out of 20, binomial test, $p < .05$) as the dogs had only one block of 80 trials to acquire the task. Similar to the mean proportion correct, there do not appear to be breed differences, in the proportion of dogs acquiring the visual discrimination.

Discussion

Pugs outperformed German Shepherds in acquiring an odor discrimination and detecting lower concentrations of the target

odorant, but did not perform differently from German Shepherds when the discrimination was visually based. We initially hypothesized that German Shepherds, a prototype breed used for scent work, would outperform a brachycephalic breed, Pugs, on an olfactory discrimination. Our hypothesis was not supported by the data: instead, the results strongly indicate the opposite. Pugs readily outperformed the German Shepherds. The differences on the olfactory task cannot easily be explained as general learning differences, general trainability, or simple motivational differences because we found no difference on a visual discrimination task. The German Shepherds readily acquired the visual task at the same rate as the Pugs.

One possible explanation, however, is that German Shepherds simply lost motivation or became "distracted" during the sessions. If this were the case, we would expect German Shepherds and Pugs to start off with similar error rates, but as the session continued, the error rate for German Shepherds would rapidly increase. Looking at the distribution of errors made by both Pugs and German Shepherds during the sessions, the error rate appears largely uniform across the entire session (see Supplementary Figure 2). In addition, a Kolmogorov–Smirnov test indicates that error distributions for Pugs and German Shepherds are not different for acquisition ($D = 0.0556$, $p = .8205$), the dilutions ($D = 0.0865$, $p = .5739$), or the visual discrimination ($D = 0.077$, $p = .7481$). This indicates that the differences in performances are unlikely to be explained by within-session decreases in motivation.

Of interest to the authors, nine of the 10 Greyhounds failed to complete the acquisition sessions. The Greyhounds could not be motivated to dig in the bucket of pine shavings, although they would take a treat from the Experimenter. Thus, although Greyhound performance was clearly different from that of the German
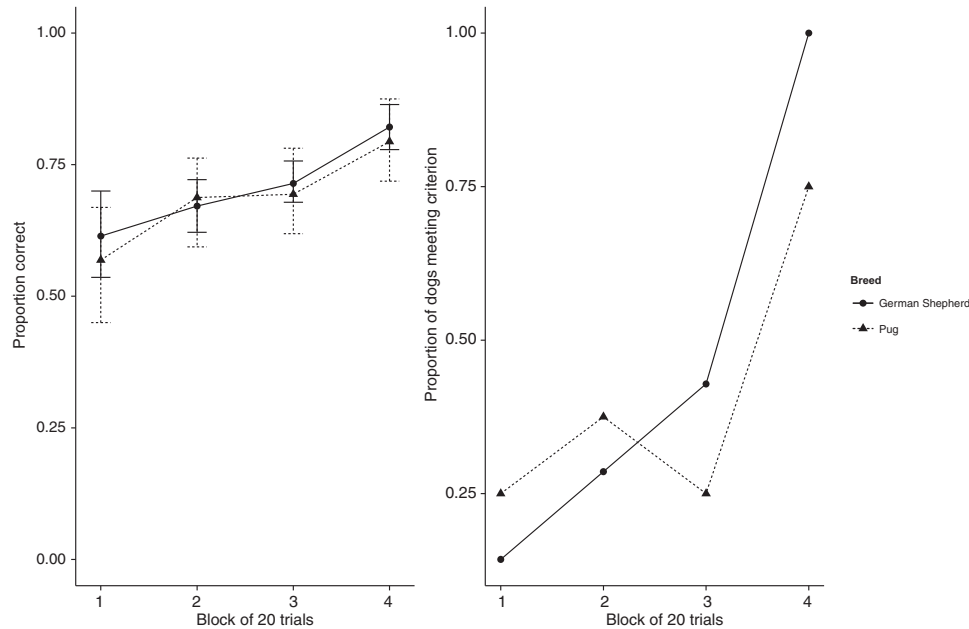

Figure 4. Visual discrimination performance. The left figure shows the mean proportion correct for each breed across sessions and error bars show the 95% confidence interval computed using a bootstrapping procedure. The right figure shows the proportion of dogs meeting a 75% criterion for the visual discrimination (German Shepherds: $n = 7$, Pugs: $n = 8$).

Shepherds and Pugs, this difference was unlikely related to olfaction, but reflects instead a motivational difference in the training.

Our comparison of German Shepherds and Pugs shows that Pugs acquired a simple odor discrimination faster, and performed better on the same odor discrimination at lower odorant concentrations. One limitation of the present study is that the dogs were not a truly random sample. Dogs were recruited based on response to one of several solicitations. Thus, the present results should be generalized carefully to the larger population. In addition, it is important to note that with more training at the lower odorant concentrations, careful selection of individuals within a breed (e.g., selecting carefully dogs bred from working lines), and careful presentation of controlled stimuli via an olfactometer, that German Shepherds may show similar performance and ultimate threshold detection levels lower to or comparable to those of Pugs. Because of the rapid nature of our dilution assessment, it is possible that the poor performance on the lowest dilution was the result of a generalization failure and not necessarily a physical inability to detect the odorant. Thus, the dilution series in the present study cannot be viewed as an assessment of threshold detection. Significantly longer training histories at more dilution steps would be necessary to identify thresholds of detection. In addition, our use of an emulsion as the diluted target odorant would make true concentration of the target odorant impossible to assess. However, the dilution series better reflects the dogs' spontaneous performance in identifying lower concentrations of the target odor. Identifying threshold detection concentrations was beyond the scope of the present project as this would have required significantly longer training times, making it prohibitive to use pet dogs. Such a study, however, would certainly be a valuable next step given our results.

Pugs' superior performance only on the olfactory and not the visual task suggests that the differences we observed are related to features specific to the olfactory discrimination task and not to common features of the visual and olfactory tasks. This suggests that the breed differences on the olfactory task are likely olfaction related. This was unexpected, given that Pugs are brachycephalic, which is associated with the rotation of the olfactory lobe and has been proposed as a possible mechanism for reduced olfaction (Roberts et al., 2010). Pugs' performance was also unexpected because of the significant shrinking of the rostrum in Pugs, which may also impair olfaction. Surprisingly, however, the effect of this reduction in rostrum size has yet to be explored scientifically. Potentially, it may have little impact on olfaction, as increasing olfactory receptor density could perhaps counteract the impact of the rostrum shrinking. These results more generally confirm the need of careful behavioral research before connections between physiology and behavior can be clearly drawn, and perhaps more careful assumptions regarding different breeds' perception of odors should be adopted.

Recently, attention has been placed on the olfactory recess, which is an area in the rear of the olfactory cavity that is covered in olfactory epithelium (Craven, Paterson, & Settles, 2010). This olfactory recess has been proposed to be critical for "macrosomatic" species, and recent computational modeling has shown how the canine nasal structure may enhance olfactory sensitivity (Lawson, Craven, Paterson, & Settles, 2012). However, the nasal cavity model published to date only used a mixed-breed Labrador re-

triever. Thus, it remains unclear how the differing nasal structure of the Pug is expected to influence olfaction.

The difference found between German Shepherds and Pugs may not necessarily be related to brachycephaly, olfactory receptor differences, or other physiological differences in the olfactory system. Instead, other behavioral differences related to olfaction could explain the results. For example, Pugs may alter their sniff pattern, which may significantly alter olfactory perception. The importance of the sniff in olfactory perception is receiving increased attention (for a review, see Mainland & Sobel, 2006). Pugs may engage in a superior sniff pattern, which could be created by a prior history of reinforcement for using olfaction, making them more likely to engage in more intense sniffing. Thus, although our data suggest that the breed difference observed is specific to olfaction, this does not imply that there have to be functional differences in the olfactory systems of the two breeds. More behavioral study is needed to define the exact nature of the breed differences in olfactory perception before we can infer the functional impact of physiological breed differences.

Our data suggest that Pugs are more readily trainable on an olfactory discrimination than German Shepherds. This would suggest that Pugs might be cheaper and easier to train for scent-detection; however, there are other limiting physical qualities to the Pug that likely restrict many toy breed dogs from being used in scent work. This may include qualities like the ability to run long distances quickly, or to climb over items in the field. Alternatively, however, this may not exclude the Pugs from all situations, and in fact, in some circumstances their small size could be an asset—such as searching in confined spaces.

At a minimum, our results suggest that it is premature to seek functional consequences of physiological and anatomical differences across breeds in the olfactory system. The hypothesis that brachechephalic characteristics, such as crowding of the ethmoturbinates or repositioning of the olfactory lobes, lead to a decrease in olfactory sensitivity (Roberts et al., 2010) is not supported by our data. Our results also indicate that attempting to interpret olfactory capability of a breed from its prevalence as working olfactory-detection dogs may also be misguided.

The choice of breeds used for scent work appears to be largely historical, and not necessarily data driven. In addition, choice of breed may be based on morphological features that are presumed to lead to more desirable behavioral phenotypes such as trainability (Helton, 2009, 2010). The present study suggests that morphological features such as brachycephaly may not be an ideal estimate of olfactory sensitivity. We conclude from our findings here that more behavioral research is needed before the function of breed specific physiological and anatomical differences on olfaction can be determined.

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