THE INFLUENCE OF GENETICS ON NEOPHOBIA: RESPONSES OF WOLVES, WOLFDOGS AND DOGS TO VISUAL STIMULI

By

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Abstract

Neophobia, the fear towards new things, is a behavioural response that enables an organism to best exploit potential resources through cautious exploration of their environment. The level of neophobia expressed by an individual is affected by many variables: experience, lineage, sociality, environmental complexity and their propensity to habituate to novelty: all of which were altered during the domestication of the dog from their wild counterpart, the wolf. To investigate how the wolf and dog differ in their neophobic response, nine animals, ranging on a genetic continuum from pure wolf to domestic dog, were introduced to three novel objects and monitored via video camera during this time. Animals of high wolf-content spent more time close to the object in exploration, had a shorter latency to approach the object and expressed significantly more nervous behaviours. This denotes that high wolf-content individuals were more attentive towards novelty than those of low wolf-content, which was a measure of fearfulness and interest in the novel object, dubbed "cautious exploration". My results suggest that individuals of higher wolf content have a genetic disposition that make them inherently more neophobic than those of higher dog content, but also show a greater interest in prolonged exploration of novelty.

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Introduction

Exploration is a vital part of an animal's survival, allowing them to gather information from their environment regarding potential resources that could enhance survival or reproduction (Dall et al. 2015). However, exploratory behaviour has risks; the potential resource or environment being explored may also be harmful to that individual (Mettke-Hofmann et al. 2006). Neophobia—a fear towards new things—allows animals to investigate novel resources more cautiously to best exploit them without suffering negative consequences (Moretti et al. 2015). Animals have a certain level of neophobia, influenced by variables such as past experiences, an organism's lineage, social structure of the species, variability of the environment and an individual's disposition to habituate to novelty (Moretti et al. 2015).

Experience is an internal factor that can affect neophobic behaviour in two ways: 1. it can reduce avoidance behaviour because the stimulus becomes less novel; or, 2. it can reduce interest in the stimulus because no further information can be gathered (Mettke-Hofmann et al. 2006). Every individual has different experiences and the stimuli they have encountered in the past will impact how they respond to novel stimuli in the future (Sih et al. 2004; Wilson et al. 1994). This is a result of cognition, an individual's ability to acquire, process, store and apply information that it has gained from the environment through their experiences (Sih et al. 2012). Novel stimuli are simultaneously categorized as potentially dangerous but also of potential use to the animal, which can cause them to behave both bold and nervous towards the stimuli.

Cognition displayed in an animal—how they acquire and apply information they have gathered—is apparent in these opposing responses to novel stimuli. This manifests as two types of trade-offs the animal undergoes when exploring novelty: speed-accuracy and risk-reward (Sih et al. 2012). Either an animal can apply a fast and risky cognitive approach to exploration with

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higher rewards if correct (but large costs if incorrect), or a slower and cautious strategy with less rewards but lower costs. Depending on past success of cognition, an animal will be more inclined to apply a similar approach in the future, which is referred to as their 'cognitive style' (Sih et al. 2012). These past experiences shape the cognitive style of the individual and result in consistent differences in behavioural tendencies, otherwise known as personality traits (Sih et al. 2004).

The other component to trade-offs is behavioural syndromes; behavioural syndromes are closely related to cognition and are consistent behaviour patterns, which manifest themselves as an individual's responses to different stimuli presented under distinct contexts (Sih et al. 2012; Sih et al. 2004). They are influenced by various, related personality traits, which are the product of experience and heritage (Sih et al. 2004). For example, aggressiveness towards others may be correlated to boldness or risk-taking in other contexts, and both could be correlated with overall activity level (Sih et al. 2004; Coleman and Wilson 1998; Oers et al. 2003). In the context of exploration, a more aggressive individual will behave more boldly with greater risk towards novelty and exhibit much higher activity (Sih et al. 2004; Coleman and Wilson 1998; Oers et al. 2003). Ergo, to aptly analyze the neophobic response of an individual, we must also analyze these over-arching correlated personality traits associated with neophobia and exploration (Verbeek et al. 1994).

Lineage, like experience, can influence the level of neophobic response exhibited by an individual (Oers et al. 2003). Behavioural syndromes are affected by genetic mechanisms, which provide a heritable component that can be passed between generations (Sih et al. 2004). Oers *et al* (2003) demonstrated this in their experiment when they collected 94 great tits (*Parus major*) from 2 different populations to investigate the relationship between risk, exploratory behaviour

and population. They used bi-directional artificial selection for "fast" versus "slow" exploring individuals over four generations to determine the heritability of personality traits (Oers et al. 2003). When exposed to a novel object, boldness, aggression, activity and risk-taking were found to be correlated between parents and their offspring, and had a large amount of additive genetic variance (Oers et al. 2003; Coleman and Wilson 1998).

This provides a strong inference that individual personality traits may be collectively coupled under natural selection and are passed down to subsequent generations (Oers et al. 2003). However, through gene-environment interactions, early experience can impact personality traits and alter the behaviour or cognition of an individual (Sih et al. 2012; Grandin and Deesing 2014). Phylogeny—the sequence of events involved in the evolution of the species—may provide the foundation for personality traits, but positive or negative experiences can alter the expression of that behaviour in the future within individuals. This causes experience to have a long-term effect on behaviour, and through selective pressures, such as natural selection, offspring gain the propensity to learn behaviours that were successful (Grandin and Deesing 2014).

Natural selection is not the only means of shaping heritable traits (Oers et al. 2003). Domestication via human-exerted selective pressure (also known as artificial selection) can alter a species' behaviour, morphology, development and physiology to traits favoured by humans (Grandin and Deesing 2014). Artificial selection can result in a large amount of phenotypic variation in a species over multiple generations (Trut et al. 2009). The species adapts not only to man, but the environment they provide as well, which alters the personality traits of subsequent generations from what was favoured in the wild, to what is favoured by humans (Grandin and Deesing 2014).

Trut et al. (2009) used the fox as a model, because they are one of the only wild canids without previously known lineages for any currently-domesticated dogs, to show the effects that early domestication had on a canid species. The initial foxes to domesticate were selected using behaviour, as it likely would have occurred with the first domestication of dogs (*Canis familiaris)* from wolf-stock. The researchers selected individuals who showed the least fear-response to handlers as breeders, to determine how many generations it would require to create a tamer (as measured by lowered aggressive behaviour when approached by handlers), more docile offspring, which are characteristics exhibited by domestic dogs (Trut et al. 2009). As selection for more tame foxes continued, the offspring also began to develop unrelated physical traits, such as floppy ears and curled tails, which are often associated with domestic dogs (Trut et al. 2009). This lead to the conclusion that behaviours associated with being tame are pleiotropic (a gene influencing two or more seemingly unrelated traits), resulting in linked selection for specific physical traits as well (Trut et al. 2009; Grandin and Deesing 2014).

Based on testing domestic foxes for exploratory behaviour in a novel environment, Trut et al. (2009) observed that selection for more tame foxes had also slowed the developmental rate of the fear response, causing it to occur approximately 3 months later than those not selected for tameness. Similar results were discovered in Hare et al. (2005)'s experiment, where captive-bred and non-captive-bred foxes were introduced to novel objects. The captive-bred foxes were selected for tameness and were far more likely to approach the novel object than non-captive-bred foxes, leading to the conclusion that the selection for more tame foxes resulted in offspring that were less neophobic (Hare et al. 2005). This shows that through the domestication process, selecting for tamer individuals resulted in offspring that were less fearful of new things in only

four generations, seemingly due to a linked syndrome between neophobia and aggressive response to novel stimuli (Sih et al. 2004).

In only four generations, Trut et al. (2009) created less neophobic fox offspring, which also exhibited some physical characteristics of domestic dogs. Though, over the approximate 10,000 years of domestication that dogs have experienced, there are even more behavioural and physical characteristics that divide them from their ancestor, the wolf (*Canis lupis*) (Scott and Fuller 1992). Behaviourally, developmentally, and even morphologically, the dog has evolved to be different than its wild counterpart, despite still being a sub-species of the wolf (Scott and Fuller 1992). The selective pressures of domestication were not the only influence on dog behaviour, but also, their changing social dynamic (Scott and Fuller 1992). This alters their species' ecology, which will affect their neophobic tendencies.

Wolves and dogs primarily differ in their social behaviour and structure, because a dog's upbringing is different than that of a wolf (Moretti et al. 2015; Scott and Fuller 2012). As puppies, dogs are often separated from their parents long before maturity, which means there is no family unit in need of pack structure during adulthood, nor is there typically a division of labour in a dog pack due to a lack of clear dominant-subordinate relationships (Boitani and Ciucci 1995; Scott and Fuller 1992). The result is that factors such as age, hierarchy and gender typically do not affect most dog behaviours (Scott and Fuller 1992). Wolves are accustomed to working together as a pack to succeed, especially in exploring potential resources and defending against danger (Moretti et al. 2015). Moreover, a wolf pack is a familial unit; individuals have incentive to act altruistically due to the inclusive fitness advantages of ensuring the survival of others who are closely related to them (Fox 1973). This difference in social structure can influence how animals can respond to novel stimuli, making them more, or less, likely to

approach objects based on their social rank, or even the presence of packmates (Moretti et al. 2015).

Moretti et al. (2015) tested how dogs and wolves would respond to novel stimuli when encountering it as individuals, pairs or packs. The researchers discovered that both wolves and dogs were less neophobic in groups or pairs, but wolves showed a greater interest in cooperating and exploring the novel stimuli (Moretti et al. 2015). However, the wolves also approached the objects more slowly and startled more easily, but also spent longer exploring the objects than dogs. This suggest that wolves are more willing to share the risks of exploration and had a higher interest in investigating novelty than the dogs, but also exhibited more fear (Moretti et al. 2015). One possible reason for the wolves' greater interest in cooperation may be their superior social learning to dogs, allowing them to utilize the information gathered by other individuals to assess novelty with fewer risks (Range and Virányi 2014). During individual trials, wolves were more neophobic of novel objects but also more willing to approach them (Moretti et al. 2015). The lack of interest in novelty exhibited by dogs may be a result of their disposition to be more attracted to familiar objects, as opposed to avoiding the object out of fear (Udell et al. 2010). Wolves, on the other hand, may have been under greater selective pressure to explore novelty by being accustomed to environments where they must be bold enough to exploit a resource, yet careful enough to avoid negative consequences (Moretti et al. 2015). However, this can differ on an individual basis depending on their habituation (the reduction, or lack of a response to a stimulus due to repeated exposure) to the environment or a set of conditions (Moretti et al. 2015).

Habituation can cause an individual to be less responsive to novelty, regardless of inherent differences in species' behaviour. Due to domestication, dogs have adapted to living in a more static (unchanging) environment, where all their resources are both predictable and in close

proximity, and they have less need to travel, or work to obtain them (Scott and Fuller 1992). This has affected the dogs' cognitive mechanisms, reducing their overall responsiveness to environmental change (Boitani and Ciucci 1995). In fact, genetic selection for more tame dogs during the domestication process may have lowered their ability to respond to these changing environments in a behaviourally flexible way (Moons 2015). The wolf however, lives in an ever-changing, complex environment where they sometimes must travel 30 miles a day in search of food and cooperate with a pack to survive (Fox 1973; Scott and Fuller 1992). These differing environments and upbringings have resulted in a lower necessity for dogs to explore and less negative consequences associated with novel stimuli (Mettke-Hofmann et al. 2006; Lord 2012). When an environment is constantly changing, or the individual is moving to different environments, it prevents them from habituating to the current stimuli (Wemelsfelder 1997). A relatively unchanging environment will also reduce the animal's ability to problem-solve and respond to differing stimulus in a flexible way (Sih and Giudice 2012; Moretti et al. 2015). Additionally, that animal would also be exposed to less novelty, which will impede their ability to transfer knowledge gained from one novel stimulus to another (Toates 1998; Singer 1985). This is known as cognitive transference; the application of previously acquired information to similar situations or objects in the future, which can cause an individual to habituate to novel stimuli that have traits previously explored in the past (Toates 1998; Singer 1985).

Dogs have not yet fully speciated from wolves, as they can still mate to produce fertile offspring (Vila' et al. 2003). The product is what is known as a wolfdog, a mix between the two species, who's behaviour depends on the random assortment of genetics they receive from their parents (Vila' et al. 2003). Wolfdogs are classified based on their wolf content; the proportion of their phenotypic characteristics that are more wolf-like than dog-like in character. This is

determined through both known genetic lineage and by phenotyping, the latter of which is a method used to assess variation in expressed traits (Saetre et al. 2004). Wolves and dogs have a near identical genome, but they do differ in phenotypic expression based on variation in alleles that are associated with different physical and behavioural traits. Through the classification of multiple expressed characteristics that are either "dog-like" or "wolf-like", hybrid offspring can be categorized along a 5-point continuum from pure wolf to domestic dog. If wolves' and dogs' genetics are the determining factor in exploratory and neophobic behaviour, then wolfdog content should reflect this trend: wolfdogs of higher wolf content should be more neophobic but also more exploratory than those of low content.

My objective is to compare the response of wolves, wolfdogs and dogs (with similar experience in captivity to control for habituation/environment) to novel visual stimuli. I hypothesize that individuals of higher wolf content will exhibit a greater neophobic response towards a novel object than those of higher dog content, but also be more willing to exhibit a prolonged exploration of the object.

Methods

Study Area and Subjects:

The study took place at Wild Spirit Wolf Sanctuary in Ramah, New Mexico (34.54484, -108.29060) over a three-month period (June to August 2016). The study area itself is a one-acre enclosure (~ 45x90m sides) that all leash-trained animals are given access to for a maximum of 120 minutes each day (if it is not currently occupied) for exercise. The study enclosure is located beside three inhabited enclosures, but individuals from those enclosures were not included in the study to avoid the use of social information—knowledge gained about the environment from observing others (Nicol 1995).

Ten subjects were selected in total, two from each respective category along the five-point wolf-to-dog categorization, as specified by the Sanctuary's classification system. The classification system is based on both genetic heritage and phenotypic expression, using 7 physical and 5 behavioural phenotypes that are inherently different between dogs and wolves (Table 1). The five categories were: pure wolf (12/12 phenotypes expressed as "wolf-like" and no non-wolf ancestors in known lineage); high-wolf content (9/12 wolf-like phenotypes and only one non-wolf ancestor in known lineage, not involving immediate parent); mid-content (6/12 wolf-like phenotypes and one parent is pure wolf, one domestic dog or both parents are hybrids); low-wolf content (3/12 wolf-like phenotypes and one parent is a hybrid and the other has no wolf lineage or both parents are mid-low content hybrids); no-wolf (0/12 wolf-like phenotypes both parents are known domestic dog lineages).

Procedure:

Subjects were selected amongst animals with similar levels of habituation to captivity, while other personality traits, such as dominance, were recorded so they could be added to the model.

Age was also recorded, due to its potential effect on an individual's behaviour. To prevent object association with the person escorting them, each subject was guided to the study area via leash and left in a 3x4m sub-enclosure for two minutes prior to being released into the study area. One of three novel objects were inside the enclosure; the same object (a 30cm square block painted with blue, non-toxic paint to make it noticeable) was used for the first trial for all individuals, allowing the initial response to a specific novel stimuli to be compared across all animals. Animals that showed a more moderate fear response in the first trial were retested two additional times, each trial separated by approximately two-three weeks. To ensure that the object placed in the enclosure was 'novel' for each subsequent counter, I used two different stimuli—a 120L garbage bin and a 1.5cm long x 2cm diameter rope, both of which were painted with the same blue paint as above—presented in block random order across subjects. These three stimuli were chosen because none of the subjects had prior exposure to similar objects in their enclosure. The individual's interaction with the novel objects were monitored via video camera for 60 minutes.

To ensure the objects were discovered by the individual within the study area, they were placed on a tarp ~20m from the entrance to the study area and were handled using polyethylene gloves as to not transfer scent. Trials did not begin until a month after the addition of the tarp, during which, all subjects visited the study area. The object and 2m radius of the tarp were washed following every trial. The fence of the enclosure and sub-enclosure were covered with tarps before any trials began in order to obstruct the animal's view of the experimenter releasing them into the study area. However, adjacent, occupied enclosures were still visible to the subject.

Trials occurred over a maximum of 90 minutes; the average period of time individuals spend in the enrichment/study enclosure on any given day, which provided the subjects with enough time to notice and interact with the object. I used a minimum 10-day interval between consecutive trials with the same individual and different objects, and access was given to all subjects to the study area without the presence of novel objects during this inter-trial time. This was to avoid the subject creating a negative association of the exercise period with the stress caused by this novelty.

Analysis:

Thirty trials were recorded in total and assessed using BORIS (Behavioural Observation Research Interactive Software—http://boris.readthedocs.io/en/latest/), a video analysis program designed to evaluate ethograms by setting parameters and keys to record behaviours. The following behaviours were recorded when evaluating the video trials in BORIS: time required to detect the object upon entering the enclosure (measured as approach within 2m, while directing a behaviour towards the object); frequency of approach; time to approach within 5m, 2m or contact with the object, whether contact was made with the object and latency for this to occur from the start of the trial; time spent investigating the object; closest approach to the object and any of the behaviours listed in Table 2 below, if directed towards the object. These expressed behaviours are relative to neophobia in canids; they signify that the subject is nervous/submissive or bold/dominant. The majority of these behaviours are expressed the same in wolves and dogs, which makes almost every expressed behaviour applicable to both.

I used a multivariate approach, Principle Component Analysis (PCA), to quantify the neophobic responses of each individual and compare them to their classification along the wolf-to-dog categorization. The PCA was conducted on 11 variables: total visits within 5m; 2m and

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total contacts with the object; total duration within 5m, 2m and contact and lastly, the total number of observed nervous and bold behaviours. I first used a General Linear Model (GLM) to determine whether type of stimulus presented affected the PC response scores, adding the ID of the subjects as a random factor to control for individual differences in response to stimuli. I then used another GLM to compare PC scores along the 5-point categorization of wolf content to test for significance in trial 1, both including and excluding dominance (determined by hierarchical rank in the individual's group or pairing) as a fixed-effect to observe its influence. In addition, a mixed-effect General Linear Model (GLM) was used to test for changes in individual response between the three trials (within-subject effect), and whether this was influenced by either wolf content or dominance (between-subject responses) of the subject. Post-hoc comparisons between groups were conducted using Fishers Least Squared Distance (LSD) analysis and a GLM to ensure the three classes of stimuli (cube, garbage bin and rope) could be grouped. All statistical analysis were conducted in R (3.3.2 R Development Core Team 2016).

Table 1. The 7 physical and 5 behavioural traits used to differentiate between dogs and wolves and categorize wolfdogs at Wild Spirit Wolf Sanctuary

Characteristics	Wolf-like	Dog-like
Physical		
Ears	Rounded ears with more fur inside	Tall, pointed ears with little fur inside
Eyes	Slanted eyes that are never brown in colour	Eye not slanted and typically brown or blue in colour
Tail	Long, bushy tail that never curls	Curled tails that come in a variety of sizes
Precaudal Spot	Precaudal spot present	Precaudal spot absent (excluding some huskies)
Chest and Legs	Narrow chest with cow-hawked hind legs	Broad chest with straight hind legs
Paws	Large paws with slight webbing between the toes	Smaller paws without webbing
Cape	Abundance of fur around the shoulder-blades	Absence of fur around the shoulder-blades
Behavioural		
Bonding	Bond at a slower rate	Bond at a faster rate
Dominance Displays	Display dominance postures frequently	Display dominance postures less frequently
Tail Posture	Tail posture signifies dominance or aggression	Tail posture signifies happiness or excitement
Vocalizations	Do not make barking vocalizations	Use barking as a vocalization
Approach	Linear approach signifies dominance or aggression	Linear approach signifies submissiveness or happiness

Table 2. The expressed behaviours of canines when nervous/submissive or bold/dominant (from Goodman *et al*. 2002)

Behavioural Set	Description
Nervous/Submissive	
Tail-tuck	Tail tucked between the legs and will often touch the belly
Crouch/Creeping	Legs bent accompanied by an arched back, providing a noticeably hunched appearance
Ears Airplane or Back	Ears held out to the side, inner surface down or lowered and folded against the sides of the head
Indirect Approach	A nonlinear approach using a meandering or curved path, often with ears back and sniffing
Pawing	An extension or waving of the paw to touch a stimulus or stroke the air in front of it
Avert Gaze	When the individual breaks eye contact, but may not involve turning the head
Jump Back	To jerk back in response to movement, or leap away
Escape	A fast trot or run to leave an area with quickened pace
Bold/Dominant	
Tall Posture	When a wolf draws itself up to its full height, arching the neck and prickling its ears
Mark Object	Urination on the object, or kicking up dirt to leave the individual's scent
Mark Radius	Urination within a 5m radius of the object, or kicking up dirt to leave the individual's scent
Sniff Object	Sniffing of the object, or its immediate radius (< 1m)
Sniff Radius	Sniffing within a 5m radius of the object, or following a scent towards the direction of the object
Tail Level with Back	Erect tail posture that is level with the back
Mouthing	To hold or manipulate with the mouth, usually repeatedly changing grip

Results

Principle Component 1 (PC1) accounted for 50.57% of the total variance shown in response to the novel stimuli in my experiment. This exceeded the value of 27.45% for PC1 expected by chance alone using the broken-stick method (Legendre and Legendre 1998). All variables with loadings of >0.33 were considered as contributing significantly to PC1, and those with >0.50 were considered as contributing strongly and those >0.70 as contributing very strongly (Ho 2006). Of the 11 variables included in the analysis, all 11 significantly contributed to PC1 (>0.33 loadings) (Table 3). The only variable that did not strongly contribute (>0.50) was the latency of individuals to approach within 5m of the object (Table 3). Total duration within 5 and 2m, total visits within 2m and total contacts, total number of bold behaviours exhibited and latency to first contact all had a very strong contribution (>0.70) to the model (Table 3).

   Positive loadings mean that higher PC1 values correspond with an increase in their respective variable (i.e. a high positive loading for duration or total contacts means that there were longer durations or a greater number of contacts). The only variables that received a negative loading were regarding latency, which means that an increase in the PC1 loading meant that the individual approached the object more quickly. The variable that contributed the most to PC1 was the total amount of bold behaviours observed and received a loading 0.20 greater than the total nervous behaviours (Table 3). The total duration spent within 2m had the highest loading, whereas the lowest was the duration contacting the object (Table 3). The opposite trend was observed for total number of visits, with total number of contacts having the highest loading and total visits within 2m contributing approximately 0.05 less (Table 3). Collectively, higher values of PC1 could be interpreted as greater levels of "cautious exploration", due to the loading's

association with more time spent close to the object in exploration, a shorter latency to begin approaching the object and the animal's expressed nervous and bold behaviours.

There was no difference in the PC1 scores between individuals in response to the three different classes of stimuli (cube, garbage bin and rope - $F_{2,12}$, P = 0.61) when controlling for individual subjects. As a result, I grouped the responses across trials as representing a general response to a novel object.

When the variation in PC1 was plotted against the 5-point categorization of wolf content: pure wolf, high-wolf content, mid-wolf content, low-wolf content and domestic dog, the result was not significant (ANOVA: $F_{4,4}$ = 4.08, p = 0.10; Table 4). However, visual analysis of the data suggested that individuals of dog, low-wolf and mid-wolf content formed one response cluster (low-wolf content), while individuals of high-wolf content and wolfs formed a second, distinctly higher response cluster (high-wolf content). When the data was reanalyzed following the grouping of animals into low vs high-wolf content, high-content animals had a PC1 score ~2 times greater than those of low content, thus having a greater number of responses and exhibiting more overall attentiveness towards the novel object (GLM: $F_{1,5}$ = 13.08, P = 0.015; Fig 1). Dominance rank (alpha vs beta) had no effect on PC1 scores ($F_{1,5}$ = 0.30, P = 0.61), nor was there an interaction between wolf content or dominance on PC1 scores ($F_{1,5}$ = 0.0022, P = 0.96).

Dominance had no overall effect on the PC1 scores across trials in the initial mixed-effects general Linear model (Mixed-effects GLM: $F_{1,4}$ = 0.43, P = 0.55), nor did interaction effects with the response across the three trials ($F_{2,8}$ = 0.14, P = 0.87). In this model, however, there was a significant effect of wolf content on trial responses ($F_{1,4}$ = 40.30, P=0.0031) and a strong trend for variation in response across the three trials within individuals ($F_{2,8}$ = 3.10, P=0.10). Dominance hierarchical rank was excluded from the final model to explore these effects,

comparing only the response of individuals across trials, and whether this was influenced by wolf content. Once dominance was removed from the model, I found a significant increase in PC1 scores across the three trials ($F_{2,10} = 4.72$, $P = 0.036$), suggesting that they may become habituated to novel objects over time and show greater exploration with subsequent exposures (Fig 2). Post-hoc analysis indicates that there was a significant increase in PC1 scores for trial 3 over both trial 1 (LSD: $P = 0.04$; Fig 2) and trial 2 scores (LSD: $P = 0.03$; Fig 2), whereas trial 1 and 2 did not significantly differ in their scores ($P = 0.78$).   Moreover, there was a between-subject effect of wolf-content (high vs. low) in all three trials ($F_{1,5} = 46.80$, $P = 0.0010$; Fig 2), with high-content animals having consistently higher PC1 values in all three trials than low-content animals. However, there was no interaction effect between the within-subject (response across the three trials) and between-subject (high vs. low content) variables, which indicated that the response across trials was parallel in all individuals, regardless of their wolf content ($F_{2,10} = 1.11$, $P = 0.366$). Dominance (alpha vs beta) had no effect on PC1 scores on its own (ANOVA: $F_{1,6} = 15.79$, $P = 0.0073$) or in interactions with responses across the three trials ($F_{1,4} = 40.30$, $P = 0.0032$), so was excluded from the final model (Table 4).

Table 3. Total percent of explained variance by PC1, as well as the contribution of 11 variables related to the attention paid to the novel stimuli to the PC1 loadings. Variables with a very strong contribution to the PC (>0.70) are in bold and italics, whereas those that contribute strongly (>0.50) are in bold.

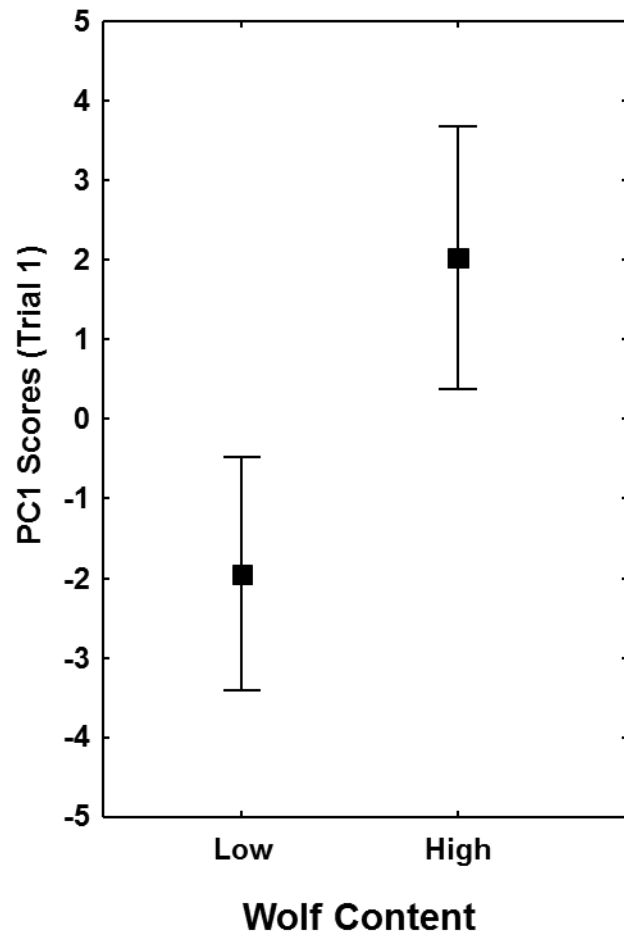| Variables	PC1
Total Visits Within 5m	**0.65**
Total Duration Within 5m	***0.76***
Total Visits Within 2m	***0.78***
Total Duration Within 2m	***0.82***
Total Contacts	***0.83***
Total Duration Contact	**0.55**
Total - All Nervous Behaviours	**0.67**
Total - All Bold Behaviours	***0.87***
Latency - 5m (sec)	-0.46
Latency - 2m (sec)	**-0.59**
Latency - Contact (sec)	***-0.73***
% Total Variation explained by Principle Component	**50.57**

Figure 1. Comparison of high and low wolf content individual's response to novel stimulus, where higher PC1 scores represent more attentiveness towards the novel object. Plots display the mean and bars display 95% CI.

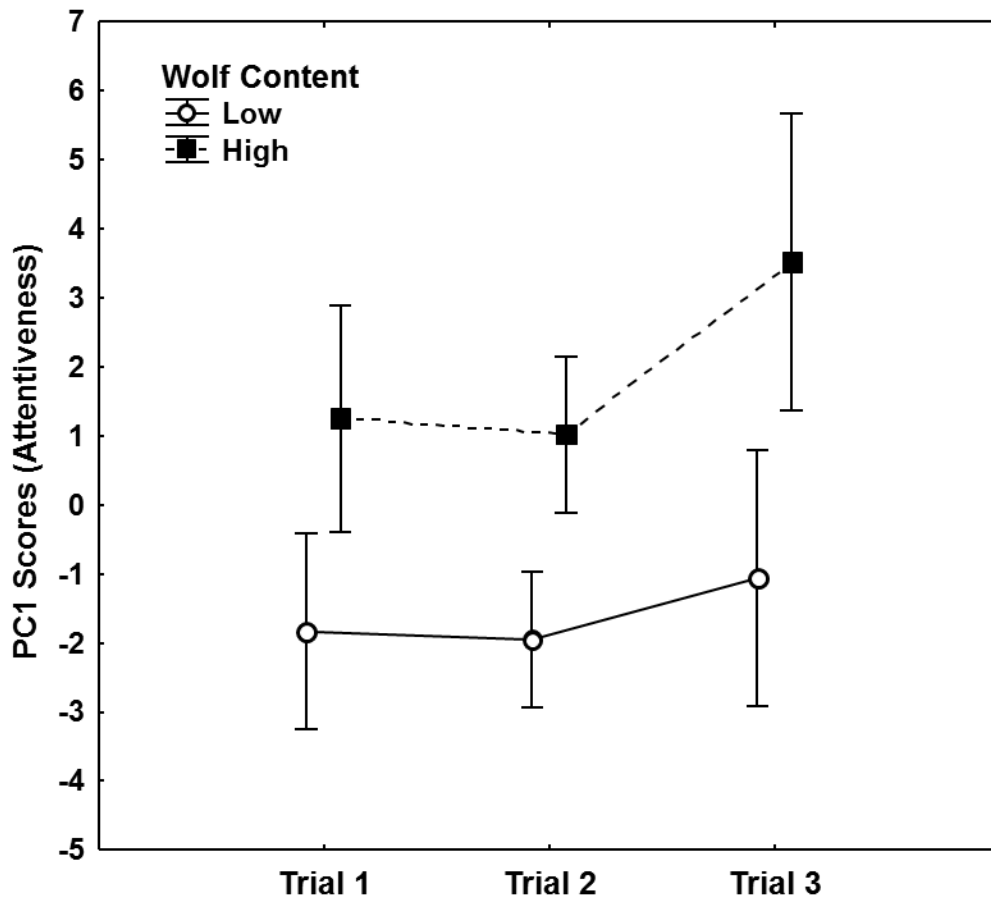**Figure 2.** Comparison of high and low wolf content individual's response to novel stimulus, between trials 1, 2 and 3 where higher PC1 scores represent more attentiveness towards the novel object. Plots display the mean and bars display 95% CI.

Discussion

My results indicate that individuals of high wolf-content were more attentive towards the novel objects they encountered; they spent a greater amount of time near the objects, approached them much earlier and expressed more fearful and bold behaviours than individuals of low wolf-content. This means that as wolf-content increased, the individual's interest in exploring the novel stimuli and their fear response towards that object also increased. Animals, of all species, express neophobic behaviour while exploring their environment for potential resources, which mitigate risk associated with unknown stimuli (Dall et al. 2015; Mettke-Hofmann et al. 2006). This fear towards new things is influenced by many variables, such as the experiences that individual has had in the past, the organism's lineage, the social structure of their species, variability of their environment and their propensity to habituate to novelty, all of which were affected during the domestication of the dog from their ancestor, the wolf (Moretti et al. 2015; Trut et al. 2009; Scott and Fuller 2012).

  The artificial selection of human-favoured traits in dogs through domestication appears to have resulted in a lack of interest and fear towards novelty in dogs in contrast to the wolf (Trut et al. 2009). It is likely that during domestication, there was selection for individuals with lower fear and aggression responses, which decreased the dogs' level of neophobic response. Their habituation to urban environments, which are more static and have easily acquirable resources with decreased risk, may have limited the negative consequences dogs have experienced during exploration of their environment (Scott and Fuller 1992). In contrast, wolves are accustomed to environments where there is a greater likelihood to encounter novel objects that are potentially dangerous (Scott and Fuller 1992; Fox 1973). My results indicate that a higher level of neophobic behaviour does not only remain in wolves habituated to captivity, but correlates with

individuals of greater wolf-content. This suggests that wolves have a genetic disposition towards responding with a higher level of neophobia than dogs and may have inherited personality traits required for surviving in more complex and dangerous environments.

Though individuals of high wolf-content consistently showed more attentiveness towards novelty, both high and low-content animals show indications of becoming habituated to novel stimuli over repeated trials. As they encountered more novel objects, all individuals began to exhibit more exploration behaviour towards them, suggesting that there may have been cognitive transference of their experiences between objects (Toates 1998; Singer 1985). Experience with novel stimuli may have been linked using transference of cues shared by the three different objects, as well and the environment they were encountered (Toates 1998; Singer 1985). All objects were painted blue, a very prominent and noticeable colour for canines, which could have been a visual trait connecting an individual's experiences in the three trials. Furthermore, the tarp that the novel object was placed on remained consistent across all trials. Individuals encountered the tarp separately from the objects and were given time to habituate to its presence over a one-month period before trials occurred, but it is possible that animals were habituating to the way the novel object was presented, as well as the novelty of the objects they were presented with.

Interestingly, wolf-content did not affect the rate of habituation towards novelty, as all individuals became more attentive in later trials. High wolf-content individuals appeared to be successively less fearful of novel stimuli as they progressed through the three trials, despite the occurrence of the objects being randomized for each individual. However, animals of low wolf-content were likely becoming more interested in novelty over time, as opposed to less fearful. The wolf and their domesticated counter-part, the dog, have experienced different selective pressures and have evolved to separate environments, which has resulted in a lower necessity for

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dogs to engage in exploration behaviour and has given them fewer negative consequences associated with novel stimuli (Scott and Fuller 1992). Dominance appeared to have little-to-no effect on the habituation, or the behaviour of wolves, wolfdogs and dogs towards novel stimuli across all three trials. This is likely because trials were conducted on individuals, not pairs or groups (Moretti et al. 2015). Dominance and submission plays a key role in social behaviour and risk-sharing, but when investigating an environment alone, the hierarchical rank of that individual does not seem to influence their attentiveness towards novelty.

My experiment was limited by a small sample size, based on the available animals who met my criteria for selection at Wild Spirit Wolf Sanctuary, reducing the power of my results. Despite this, though, I still found a strong effect of wolf content on responses of subjects to stimuli. Additionally, over the course of my three trials, the animals appeared to habituate to stimuli other than the novel object, which may have exaggerated their increase in attentiveness. The proximity of the study area to other, occupied enclosures may have also influenced the behaviour of the individuals in my experiment, due to the audience effect. Even so, the occupants of these enclosures remained constant and were standardized across all trials.

I conclude that, when introduced to novel objects, individuals of high wolf-content exhibited a greater neophobic response and a greater willingness to explore the objects for extended periods of time. This increase in neophobia was not exclusive only to wolves and dogs, but was also present in the differing contents of their hybrid offspring, which provides a strong inference that there is a heritable component to neophobia. The results of my study could further the evolution of domestication and could be used to understand how changing environments, habituation, cognitive style and experience influence canine behaviour. This may also bring credibility to the classification system of wolf hybrids using physical and behavioural traits, and the use of

wolfdogs in dichotomous scales to distinguish differences between wolves and dogs. The interesting results on habituation to novelty, possibly due to cognitive transference, may also merit further experimentation and insight into the cognition of canines.

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