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The Use of Bloodhounds in Determining the Impact of Genetics and the Environment on the Expression of Human Odortype

ABSTRACT: Bloodhounds are used to trail fleeing felons and missing persons. In order to start a trail, the dog must be presented with a person's scent. There are many hypotheses on what a bloodhound smells while trailing. The present study attempts to identify whether human scent is genetic, and if it is influenced by one's environment. Bloodhounds trained in human scent discrimination were used to differentiate between monozygotic twins, related and nonrelated persons, living together and apart. The first test required the dogs to run blind trails after being presented with the scent of one person in the pair, while the opposite person was hidden. The second test allowed the dogs to trail one person in the pair after both people were hidden. Results appear to demonstrate that bloodhounds rely heavily on genetic cues when differentiating between people. Environmental cues do not appear to significantly aid the bloodhound in scent discrimination.

KEYWORDS: forensic science, bloodhound, canine, odortype, major histocompatibility complex (MHC)

Studies have been conducted in order to demonstrate that various breeds of dogs have the ability to discriminate human scent. However, few investigators have attempted to analyze the origins of human scent. One such study looked at the possible genetic component of scent by having a dog attempt to distinguish between identical twins (1). The dog was not able to distinguish between the scent of one identical twin's body odor and a handkerchief scented from the other twin after it had been laid out in an array of handkerchiefs which were scented by various people. When the same dog was given the opportunity to track a pair of identical twins hidden in a field, the dog was successful in finding the correct twin. A second study using tracking dogs to distinguish between articles scented by identical twins appears to indicate that the dogs were able to pick out the twin's matching article if environmental cues were different (2). Both of these studies have concluded that genetic factors appear to be regulating metabolic processes which lead to the effusion of distinctive body odors for every individual.

Dog handlers have made the assumption, based on anecdotal evidence, that every person's scent is different from another's (3). In studies conducted under controlled conditions, scientists have demonstrated that other breeds of dogs used for police work, beside the bloodhound, are able to match scent from articles with the person who had previously touched them (4,5). If the assumption is indeed correct and a person's scent is as individual as a fingerprint, then chemicals that make up scent are most likely to be controlled by one's genetics, and these genes must be highly polymorphic. The major histocompatibility complex (MHC) is the most polymorphic set of alleles in the human genome (6). Studies have demonstrated that MHC is involved in regulating the production of transmembrane proteins used by the immune system to identify self from nonself thereby producing a vast diversity of these transmembrane proteins across the population (7). Further studies have proposed that the MHC and its phenotypic expression of antigens may account for the differences in individualized odors (8,9).

The α_1 and α_2 chains of the MHC form the two side walls of the transmembrane protein; while a β-pleated sheet forms the base in a U-shaped configuration (10). The unique shape of this protein can bind a *milieu* of volatile chemicals. The individual "cocktail" of volatiles is then transported through the bloodstream by the protein to the renal system, sweat and salivary glands, where the protein is degraded (11). The volatile chemicals are released from the protein, eventually being excreted onto the body's surface. The body's excreted volatiles are thought to confer an individual's phenotypic odor expression also referred to as their odortype (12).

Law enforcement organizations have used a variety of dog breeds, including the bloodhound, to trail people using articles that contain human odortype, thereby supplying vital information which may lead to an arrest, conviction, and imprisonment (3). In situations involving real-life police scenarios, bloodhounds have been required to trail a person into their family environment, where the dog must discriminate between family members (e.g., two siblings or a father and a son) to make a correct find. If scent is individual, then the following question can be posed. Is the bloodhound's ability to differentiate between human odortypes based on genetic control? If so, then (1) can the bloodhound differentiate between persons who are closely related? and (2) is the dog capable of distinguishing between monozygotic twins? The next question that should be raised is; does the environment play a role in altering a person's scent? The present study was designed to investigate whether bloodhounds can differentiate between humans based on their genetics and whether environmental cues can influence odortype.

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TABLE 1—*Demonstrates number of dogs that performed better than chance.*

Groups	Number of Dogs	Sets of People Trailed	Test 1	Test 2
Twins together	13	9	0	3
Twins apart	9	9	1	5
Related together	13	12	10	9
Related apart	12	10	12	12
Nonrelated together	13	14	13	12
Nonrelated apart	9	12	9	9

Methods

Animals

The study was conducted over a 16-month period utilizing a total of 13 bloodhounds. During the course of the study some dogs were not included in the results due to reasons that include death of three of the dogs, as well as some dog/handler pairs were busy with criminal investigations and could not attend. The number of dogs for each study is indicated in Table 1. In brief, all dogs began their training before 6 months of age with most starting at 47 days old. The dogs were all trained using the methods described by William Tolhurst (13). The Tolhurst method employs a "hide and go seek" type of trailing scenario. The dogs are taught to associate the scent on an article with that of an individual. Eventually the dogs learn to discriminate between the scent on the article, and multiple scents laid down on the ground in order to find a single person. The bloodhounds used in the present study were at least 18 months of age, with the oldest dog at 5 years of age (average age 3.4 years). All bloodhounds worked for law enforcement and/or search and rescue agencies. Each bloodhound had been previously trained to identify a negative versus positive trail.

A trail is considered negative after a scent is presented to the bloodhound and the dog cannot match the scent on the article to any scent located on the ground. The article scent is collected from an individual who is known to have never been in the area in which the dog will be trailing. If the dog performs the negative correctly he or she will not trail. The dog will typically stand in place or circle the immediate locale briefly, and then stop, thereby letting the handler know there is no trail, and the scent is a negative. During the current study, if any dog trailed off of the negative scent, that dog was later eliminated from the data.

A positive trail is distinguished from a negative when the bloodhound accurately matches the smell of an individual on an article with a trail laid by the same person. During the testing, the handlers were asked to judge whether their dog presented them with a negative or positive trail and whether the final identification was correct or not. The final identification or "tag" was distinguished by the handler, as each individual dog may present their tag in various ways. For example, one dog may jump on the person they are identifying while another dog may sit in front of the person, yet another may place a paw on the individual.

Scent Collection

All scent was collected by the protocol previously described (14). In brief, a scent transfer unit (STU-100) was used to move scent from the subject being trailed onto a gauze pad (5″ × 9″, Johnson & Johnson, Skillman, NJ). The STU-100 is a low-powered vacuum device devised for scent collection. One day before the experiment, scent was collected from each person using the STU-100. The scent was placed in a zip-loc baggie, and kept refrigerated at 4°C until the morning of the experiment. The bags containing the scent pads were marked with shapes in lieu of names in order to carry out a double-blind study.

Persons Living Together

People who had been living together were used for the first set of trails. These trail layers fell into three different groups, nonrelated, related, and monozygotic twins. There were 14 nonrelated pairs, 12 related pairs (siblings and parent–child combinations) and nine pair of twins. Each pair of trail layers lived together for more than 1 year and was required to participate in a strictly regulated lifestyle for *c*. 1 month before the experiment. The trail layers were asked to coordinate a long list of items so that both persons in the pair were consuming and using the same products. These items included all food consumed, all deodorants, cosmetics, hair sprays, various types of soaps, including soaps used to wash their clothes. DNA testing at no less than 10 marker regions was conducted on all twin pairs in order to verify they were monozygotic. The average ages of the trail layers were 22.4 years for the nonrelated pairs, 26.7 years for related pairs and 25.8 years for monozygotic twins.

Persons Living Apart

Trail layers for the second protocol were selected based on the fact that they had not lived together for at least 1 year or more. There were 12 nonrelated pairs used as trail layers, along with 10 pairs who were related (siblings and parent–child relations) and nine pair of monozygotic twins. During the testing of nonrelated and related pairs, 12 and 13 bloodhounds were utilized, respectively. Eleven bloodhounds were used to trail the monozygotic twins living apart. Only one pair of twins was used in both experiments 1 and 2. This pair of female twins married and moved apart right after the first experiment. They had not been living together for 12 months when they participated in the second experimental protocol. All of the other pairs of twins had not lived with each other for at least 5 years. One pair had not lived together for over 30 years (average length of time living apart was 10.2 years).

Experimental Design: Test 1

On the day of the experiment the trail layers gathered at a local regional park. All trails were run on grass. The park was occupied by many individuals who were milling around in close proximity to the testing zone. However, no one but the dogs, handlers and workers were permitted into the area where the trails were laid. No handlers or dogs were allowed on the scene before the start of the dog's trail. One person of each pair (person A) was asked to walk a path marked out for them on a map. Person B did not enter the field where these tests were being conducted. Person B was no less than 1/2 mile away from the testing site and had never been to the field before the day of the tests.

Person A was given instructions to walk *c*. 200 yards and then hide behind a tree or similar structure (Fig. 1). After the trail layer was well hidden, the handler and bloodhound were brought to the starting point where the handler presented his dog with a scent pad. Unbeknownst to the handlers, each was given the scent pad of either person B or a negative. No handlers were aware of who their trail layer was or where he or she was hidden. The only information given to the handler was the direction of travel and point last seen. The handler was also not aware that the scent pad given to them could cause their dog to present a "negative" trail.

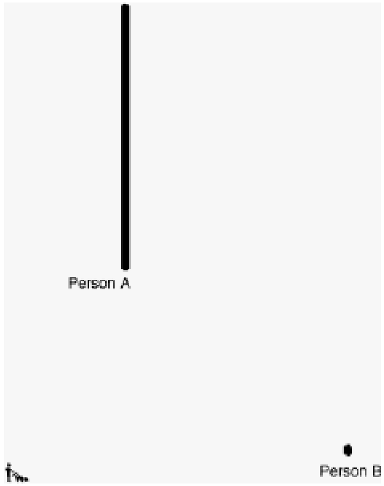

FIG. 1—*During Test 1, trail layer A was asked to walk into a field and hide behind an object. The dog was presented with the scent collected from person B in the pair. The dog was required to perform a negative trail to be "Correct."*

All trails were run within 60 min of the trail layer walking their course.

Experimental Design: Test 2

Test 2 utilized the same pairs of trail layers as Test 1 and was carried out on the same day, but in a different location in the park. Again all trails were run on grass, however, this time both trail layers in the pair walked the trail. Person A and Person B walked side by side from the starting point for *c*. 100 yards. After 100 yards they split from each other forming a Y-shaped pattern. They continued walking for *c*. 50 yards and then hid behind trees or similar objects out of sight of the dog and handler (Fig. 2).

Once the trail layers were in place the handler and dog team were brought to the scene and given the scent pad of either Person A, Person B, or a negative control. The scent pads were randomly picked by the experimenters and given to the handler to present to the dog. The dogs were given the command to trail and the trail was terminated when the handler indicated his or her dog was finished trailing. If a dog was not able to present a negative correctly, that dog was eliminated from the findings for that day only.

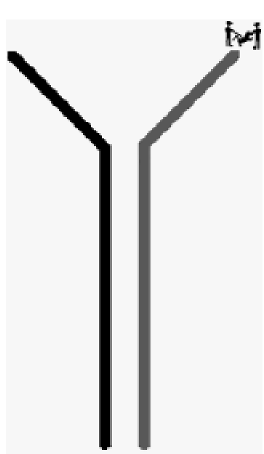

FIG. 2—*During Test 2 both people in the group walked out into a field in a "Y" formation and hid behind an object. The bloodhounds were presented with the scent of one of the two persons in the pair and asked to differentiate between the two trail layers.*

Statistical Analysis

The dog's trail during Test 1 was considered "Correct" if the dog presented a negative and was given a score of 3. The dog's trail was scored with a 2, "Undecided," if the handler indicated the dog ran a positive trail, but would not identify the trail layer. The dog's trail was considered "Wrong" when the handler indicated the dog made a positive find, when in fact the dog should have presented a negative trail. These trails were given a score of 1.

During Test 2, the trail was considered "Correct" when the handler indicated their dog made a find and the find was the right person in the pair. A "Correct" trail was scored with a 4. If the dogs ran a positive trail, but did not identify the correct trail layer, the score was a 3. A trail was determined as a positive trail with a wrong identification if the dog conducted a positive trail, but in the end identified the wrong person in the pair. These trails were given a score of 2. Finally the trail was considered "Wrong" when the dog presented the handler with a negative that should have been positive and given a score of 1.

A goodness of fit test was used to demonstrate the dogs that performed better than chance. A probability of $p < 0.05$ was considered statistically significant. The Wilcoxon signed-rank test was used to test for differences between experimental groups.

Results

Test 1

During Test 1, the bloodhounds were presented with the scent pad of person A while person B was hidden in the field. The dogs were required to differentiate between the scents of two persons when only one person of the pair was present. For the dog to be "Correct" it had to present its handler with a negative trail. Monozygotic twins appeared to be the most difficult group for the bloodhounds to differentiate correctly. There were no dogs that were able to perform better than chance when trailing the twins who lived together (Table 1). When trailing twins who lived apart for a year or more, there was only one dog out of nine that performed better than chance. There was no significant difference between the performance of the bloodhounds trailing twins living together or apart (Fig. 3).

There was however a dramatic change in the overall performance of the bloodhounds when given the opportunity to trail related people who lived together. Out of 13 dogs, 10 performed significantly better than chance ($p < 0.05$) (Fig. 4). When testing the bloodhounds on the related pairs who lived apart all 12 bloodhounds performed better than chance. When a comparison was made between related living together and apart there was no difference in the dog's ability to perform a correct negative trail.

Thirteen bloodhounds were used to trail nonrelated people living together and all 13 dogs were able to perform a negative trail significantly better than chance. There were nine bloodhounds used to trail nonrelated people living apart and again all the dogs performed significantly better than chance. When comparisons were made between related living together and apart, the dog's ability to carry out a negative trail was not significantly different.

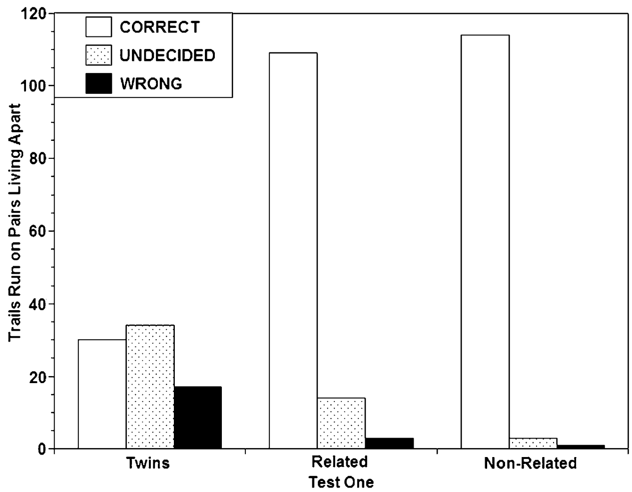

FIG. 3—*Comparison of trails during Test 1, only one person of the pair that lived together hidden in the field.*

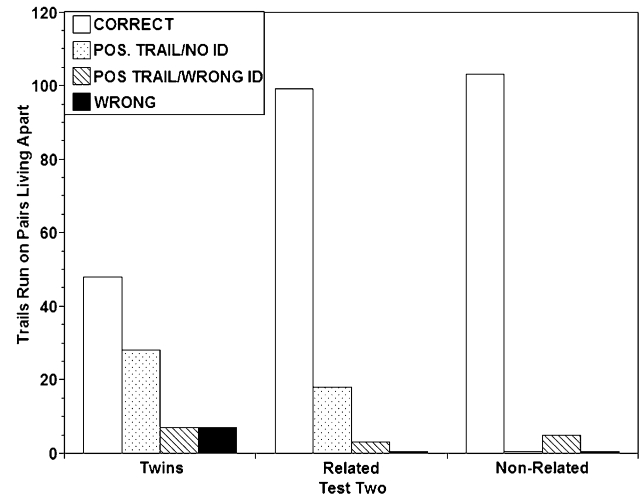

FIG. 5—*Trails for Test 2 conducted with both people in the pair living together hidden in the field.*

Test 2

During the second protocol, both people in the pair walked through a grassy field and hid behind an object. The scent from one person in the pair was picked at random and presented to the bloodhound. The data established only three bloodhounds were able to perform better than chance when trailing monozygotic twins who lived together. Five out of nine dogs were able to perform better than chance after trailing twins living apart. There was no significant difference in the dogs' ability to correctly trail and locate twins living together versus apart (Fig. 5).

After trailing pairs in the related group, there was again a significant increase in the dog's ability to find the correct person in the pair. Of the 12 bloodhounds used to trail the related living together group, nine dogs performed better than chance. Twelve out of 12 dogs identified the correct person in a pair of the related living apart group. When comparing those living together and apart there was no significant difference in the performance of the dogs.

Thirteen dogs trailed the nonrelated living together group. Of the 13 dogs, 12 performed better than chance, identifying the correct person in the pair. Nine dogs were used to discriminate between nonrelated pairs living apart. All nine dogs correctly identified the trail layer they were scented on. There was no difference in the performance ability of the dogs trailing nonrelated living together and nonrelated living apart (Fig. 6).

Weather

Measurements were taken during each trailing session. The weather was fairly consistent during each of the trails (Table 2). There did not appear to be any affect of weather on the overall performance of the dogs.

Discussion

The results of the present study demonstrate that trailing and differentiating between monozygotic twins, compared with pairs of related and nonrelated individuals, is problematic for bloodhounds. The data also suggests the discriminatory capabilities of the bloodhound seem to greatly depend upon a person's genetically derived odortype. Nevertheless, environmental signals may

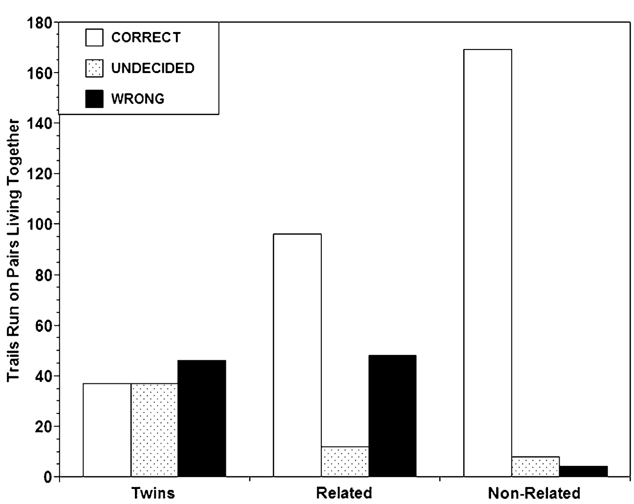

FIG. 4—*Comparison of trails during Test 1, only one person of the pair that lived apart hidden in the field.*

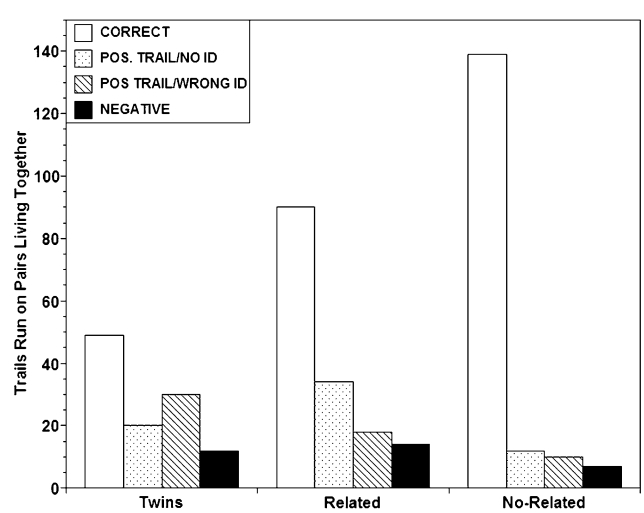

FIG. 6—*Trails for Test 2 conducted with both people in the pair apart together hidden in the field.*

TABLE 2—*Weather conditions during each testing.*

Groups	Temperature (°F)	Humidity (%)	Wind Speed (mph)	Time (hours)
Twins together	60	60	2.5	09:25
Twins apart	65	45	2.0	09:00
Related together	70	53	2.5	09:10
Related apart	73	47	5.0	09:00
Nonrelated together	74	60	2.0	09:30
Nonrelated apart	75	37	2.5	10:00

play a role in furnishing cues for bloodhounds attempting to differentiate between people who have very similar genetics, as in the case of monozygotic twins. These data would seem to indicate that the bloodhound is able to successfully apply a multifactorial approach while trailing people, utilizing both genetically derived odortype and environmental indicators.

Test 1 was designed to demonstrate whether an individual's odortype was unique enough, so that when the scent of one person was presented to the dog they could discriminate it from the scent of a second person. The data suggests the bloodhound was not able to differentiate between the scents of identical twins, whether they lived together or apart. Nevertheless, when trailing related people, the dog's ability to differentiate improved significantly. The study also demonstrated that people who are not related are less problematic for the bloodhound to differentiate between. These data would suggest that if the genetics of two people are different enough, then the dog does not need to utilize any other signals that may be provided by the environment in order to trail.

Monozygotic twins were also used as test subjects in a previous study in order to ascertain whether dogs track people based on genetic or environmental cues (1). In this prior study, dogs of various breeds were given the scent of one twin, and then asked to retrieve a handkerchief scented by the second monozygotic twin from among a group of handkerchiefs. These handkerchiefs had been scented by an assortment of people. The dogs in the study appeared to have no hesitancy in selecting the handkerchief of the opposite twin in the array. If handkerchiefs of both twins were placed within the array, the dog would select whichever handkerchief it came to first. The author concluded that dogs would accept the odor of the opposite identical twin in the absence of the correct odor. This previous study is consistent with the present findings wherein the bloodhounds were observed to perform "Wrong" trails more often than "Correct" when trailing monozygotic twins. In other words, the bloodhounds trailed after the hidden identical twin when they were presented with the scent of the opposite twin instead of presenting a negative trail.

In comparison with Test 1, Test 2 had both twins hide in a field, and the bloodhounds were given a chance to differentiate between the two scents. Although overall performance for trailing twins was better when compared with Test 1, the dogs took a much longer time to make a decision in Test 2. After being presented with the scent pad, many of the dogs ran towards one of the twins. This was not necessarily the correct twin, but after having smelled a second person at the "Y" of the trail, the dog would then run over to the second twin and smell him or her as well. The same dog may have run back and forth several times before making a tag, or in some instances just sitting down and baying loudly in frustration. The dogs showed signs of distress, agitation and confusion. The bloodhounds never exhibited confused behavior while trailing related and nonrelated pairs, living together or apart. The findings suggest that bloodhounds may use genetically derived odortype as its major source of scent while trailing. The more genetically similar two people are, the more difficult it is for the dogs to tell the difference. The similarities between two people may force the dog to rely on environmental cues for scent discrimination and trailing. In the same vein, one study suggests that odortype is not solely based on genetics, but may also be altered by varying nongenetic factors, such as environmental signals (15). These environmental cues, however, do not appear to outweigh the importance of the genetically derived scent being exuded from the person when a bloodhound trails.

Environmental factors have been suggested to play a large part in the dog's overall ability to trail. Prior research was performed using monozygotic twins to test this assumption. Dogs of various breeds were presented with the scent of one monozygotic twin after both twins had worn t-shirts for 24 h. The dogs were given the scent from one twin and asked to pick the t-shirt of that same twin out of an array of other scented t-shirts (2). The dogs were not able to differentiate between the t-shirts of the twins who lived together, and would pick whichever twin's t-shirt it came to first. However, when the dogs were given the smell of one twin, out of a pair who had been living apart, the overall performance of the dogs increased. The bloodhounds in the present study demonstrated a greater degree of uncertainty when attempting to differentiate between the smell of twins, and more so if they lived together versus apart. One suggestion for the slight improvement in the bloodhound's ability to distinguish between groups living together and apart may be that environmental odors do play a small role in scent discrimination. The ability of the dogs to distinguish between people living together and apart, in the present study, was not significant, and the data suggests the dogs rely mainly on genetically derived odortype in order to trail and scent discriminate.

Over 25 years ago, Lewis Thomas suggested: if human's odortype is genetically controlled, then one should be able to train dogs to cross-match humans for organ transplants (11). Other experimenters have also suggested that scent may be genetically controlled and dogs, therefore, should not be able to distinguish between the scents of monozygotic twins (16). Several studies have been carried out in order to examine the difference in odortypes in both animals and humans (17–19). These studies have attempted to ascertain whether there is a correlation between an individual's odortype and their genetically derived MHC, which is used for determination of organ histocompatibility. MHC demonstrates considerable polymorphism, and is the main contender of an individual's genetic scent odortype (15,20). Studies have established that MHC is used as a chemosensory signal in order to identify an individual by his or her phenotype. MHC odortypes have not only been well documented in mice and rats, but also in humans (9,12,20). Studies have also demonstrated that various animals, as well as humans, can smell and recognize their own kin, and this recognition is most likely determined by the MHC genes (21,22). Changing a single base in the genetic code of MHC is thought to be able to alter an individual's MHC odortype (10). The slight alteration, and the extreme polymorphism of MHC, may account for the ability of the bloodhound to differentiate between individuals in a population. The more changes in base pairs, the greater the alteration in odortype, and the easier it is for the dog to scent discriminate.

The question then is: "What is the dog actually smelling as it runs along sniffing the ground?" Studies to date have put forward two potential hypotheses. The first hypothesis suggests that an individual's MHC reflects the immune response against one's bacterial flora on the skin, urinary tract and gut. Therefore, a person's unique MHC would correspond to one's own distinctive bacterial flora, and that flora would not change over time (10). A study on germ-free mice have determined they can still be distinguished by their odortype, thereby suggesting that one's unique odor is not based on bacterial flora (23). A second suggested hypothesis states that an individual's unique MHC antigens carry volatile compounds that can then be transported to the urine, sweat and/or saliva (24,25). This genetically specific "cocktail" of volatile compounds is released from degraded MHC onto the body's surface and given off as scent that can be detected by the bloodhound.

In contrast to these hypotheses, an earlier study suggests that an individual does not have one unique scent, but that a person's scent varies depending upon where on the body the scent was collected (26). For instance, scent would be different if it was collected in the crook of the elbow versus collected from the palm of the hand. Current research on odortypes and MHC would be directly opposed to these findings as an individual's MHC would not be unique in different body parts. Also, in the same research, a single bloodhound was used, and this dog was able to perform better than chance. Our research would suggest that bloodhounds can identify a person by their individual odor, and that odor is based mainly on genetics, which is uniform throughout nucleated cells.

Conclusion

After presentation of bloodhound testimony in court, jurors are given the following instructions, "Dog tracking evidence is not by itself sufficient to permit an inference that the defendant is guilty of the crime. Before guilt may be inferred, there must be other evidence that supports the accuracy of the identification of the defendant as the perpetrator of the crime" (27). Even though bloodhounds have immense olfactory sensitivity, they are just animals who have been taught to play the game of hide and seek. It is up to the handler and law enforcement personnel to translate canid behavior into court acceptable evidence. The results of the present study lend credibility to the bloodhound's ability to trail and discriminate between various people using genetically derived odortype, as well as possible environmental signals.

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