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Behavior Genetics of Canine Aggression: Behavioral Phenotyping of Golden Retrievers by Means of an Aggression Test

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Molecular genetic analysis of complex traits such as aggression strongly depends on careful phenotyping of individuals. When studying canine aggression, the information provided by the owners of the dogs is often not detailed and reliable enough for this purpose. Therefore we subjected 83 golden retrievers, both aggressive and nonaggressive individuals, to a behavioral test. These tests were analyzed with help of an ethogram, resulting in a behavioral profile for each of the dogs. In this article three methods are described of converting these profiles into a measure of behavioral phenotype. The usefulness of the methods is evaluated by comparing the test results with information provided by owners. Moreover, the hypothesis underlying all these methods, that a lowered threshold for aggressive behavior in general is present in the dogs, is also evaluated. Future research will need to reveal whether the methods meet the high standards that are necessary for studying complex traits.

KEY WORDS: Dog; aggression; behavioral test; phenotyping; ethogram; genetic basis.

INTRODUCTION

Biting incidents with dogs pose a considerable problem in countries all over the world. In the Netherlands, about 240 people a year are hospitalized as a consequence of dog bites (Mulder, 1991; Schellart and Den Hertog, 1998; Toet and Den Hertog, 2000). Not surprisingly, aggression is a common reason for euthanasia of dogs (Mikkelsen and Lund, 2000). Studying the etiology of canine aggression is therefore important for both human and canine welfare (Hunthausen, 1997; Rossman *et al.,* 1997; Rusch *et al.,* 2000). Canine behavioral disorders are also interesting because they can serve as a model for human mental disorders. Dogs might represent a more valid model for humans than rodents because dog behavior is more similar to human behavior: both dogs and people can be regarded as predators that cooperate with group members (Overall, 2000).

Aggression is a complex trait in any species: it is under polygenic control and environmental factors play a role in its development (Enserink, 2000; Mackenzie *et al.,* 1986; Tecott and Barondes, 1996). The nature, relative importance, and interaction of these genetic and environmental influences are poorly understood. Our search for the etiology of canine aggression focuses on genetics. The number of different mutations responsible for variation of aggressive behavior within a single breed is expected to be low because genetic variation within dog breeds is limited (van Oost *et al.,* 2002). We therefore chose to study a single dog breed: the golden retriever. Most golden retrievers are friendly pets, but some of them are very aggressive (Edwards, 1991; Galac and Knol, 1997; Heath, 1991; Knol and Schilder, 1999). Because aggressive behavior seems to occur more often in certain golden retriever family groups (Knol *et al.,* 1997), it is likely that a genetic cause is involved. This combination of an expected genetic basis and a variation in the tendency to behave

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aggressively within the breed, make aggression in golden retrievers a very suitable subject for a study on the genetic basis of aggression in dogs.

Molecular genetic analysis of complex traits strongly depends on careful phenotyping of individuals. The *Diagnostic and Statistical Manual* (DSM) and the *International Classification of Diseases* (ICD) are often applied in studies on the genetic basis of human mental disorders, but no such instrument is available for studies on dogs. In some studies on canine behavior problems, questionnaires for dog owners have been applied. However, owners are not always skilled in observing behavior and using their opinions might lead to biased results (Galac and Knol, 1997; Hart, 1995; van der Borg *et al.,* 1991). Moreover, the information provided by owners is likely to reveal only a limited number of phenotypic classes (e.g., "aggressive" and "nonaggressive") while a more detailed classification might be necessary for molecular genetic studies. We therefore study the possibilities of using a behavioral test as more objective and detailed method of assessing the behavioral phenotype of a dog. Several canine behavioral tests that could be used to this end have been described in literature. van der Borg *et al.* (1991) reported on a test for dogs in animal shelters, which aimed to prevent bad matches between new owners and dogs. With their test they correctly predicted 75% of the problem behaviors that a dog would show in the future. Netto and Planta (1997) published a test that could be used for excluding aggressive individuals from breeding. In 43 subtests the aggressive tendencies of the dog were evaluated and they concluded that their test was a useful instrument for assessing these tendencies. Apart from these two tests, numerous other tests for dog behavior exist, but few of these were scientifically validated. We chose to use a shortened version of the test described by Netto and Planta for phenotyping golden retriever behavior.

The test can only be useful for genetic studies if the variety of behaviors observed during the test are translated into a certain measure. Two main approaches are possible for this translation, each with its specific underlying hypothesis about the etiology of aggression. The first approach adds behaviors observed during different subtests and does not take into account that the subtests offer different types of stimuli. Here, the underlying hypothesis is that the golden retrievers have a lowered threshold for aggressive behavior under various circumstances. This hypothesis is supported by Netto and Planta (1997), who suggested that highly aggressive behavior during their test was the result of a genetically based tendency for aggression. The second approach analyses dog behavior during classes of subtests with similar stimulus situations. The hypothesis underlying this approach is that aggression can be subdivided into classes based on the nature of stimuli eliciting the behavior and that these different classes are controlled by different genetic mechanisms. Literature provides some evidence for this assumption (Blackshaw, 1991; Borchelt, 1983; Moyer, 1968; Popova *et al.,* 1993; Voith, 1984; Wright and Nesselrote, 1987). The nature of the genetic basis of aggression is still poorly understood; thus it is not clear which of the two approaches is the best.

We analyzed the behavior of 83 golden retrievers, 59 aggressive and 24 nonaggressive, during the test with help of an ethogram. Both of the approaches mentioned above were used, but they will be presented in two separate papers. This first paper is based on the hypothesis that a lowered threshold for overall aggressive behavior is present in the dogs. Therefore the results presented here are all based on additions of data stemming from different subtests. Within this approach, there are again various ways of converting the test results into a measure of behavioral phenotype, three of which will be discussed. Although the owner's story was considered to be moderately reliable, the usefulness of these methods was evaluated by comparing the calculated phenotypic measures with owner-provided information about the history of the dogs.

METHODS

Subjects

The study group consisted of 83 golden retrievers, 55 of which were purebred with a pedigree. All goldens were privately owned dogs, and the majority (82%) still lived with their first owners. There were 49 males (18 castrated) and 34 females (16 castrated) in the study group. The mean age of the dogs at the time of testing was 3.3 years; three were juveniles (8 or 9 months), 8 dogs were subadults (9–18 months), 67 dogs were adults (18 months–7 years), and 5 were old dogs (7 years or older). Fifty-three of the 83 dogs were referred to behavior experts at the Utrecht University Companion Animal Clinic because of their aggressiveness. We consequently traced 30 family members (mainly siblings) of a number of these goldens and, although none of them had ever actually bitten a person or another dog, 6 of these relatives showed a problematic level of aggressiveness according to their owners. In none of the dogs was a medical problem likely to be the origin of the aggressive behavior, and none of the dogs received medication.

Questionnaire

A questionnaire for the owners was designed to gather information about both the history of their dog and characteristics of its aggressive behavior. Based on this information, an artificial classification of the dogs was made: they were classified as "owner-acknowledged nonaggressive" if the owner declared that the dog had never attacked, bitten, or showed excessive growling behavior toward either a dog or a person. All other dogs were classified as "owner-acknowledged aggressive."

The Aggression Test

The aggression test consisted of 22 subtests. The majority (19) of these were selected from the Netto and Planta test (1997) because they proved to have high aggression-eliciting power. Two less threatening subtests (subtests 4 and 5) were also included to let the dog acclimatize to the testing room and to make the test more acceptable for the owner. A new subtest, using a dog mask (subtest 21), was also included. All tests were performed in the facilities for dog research at Utrecht

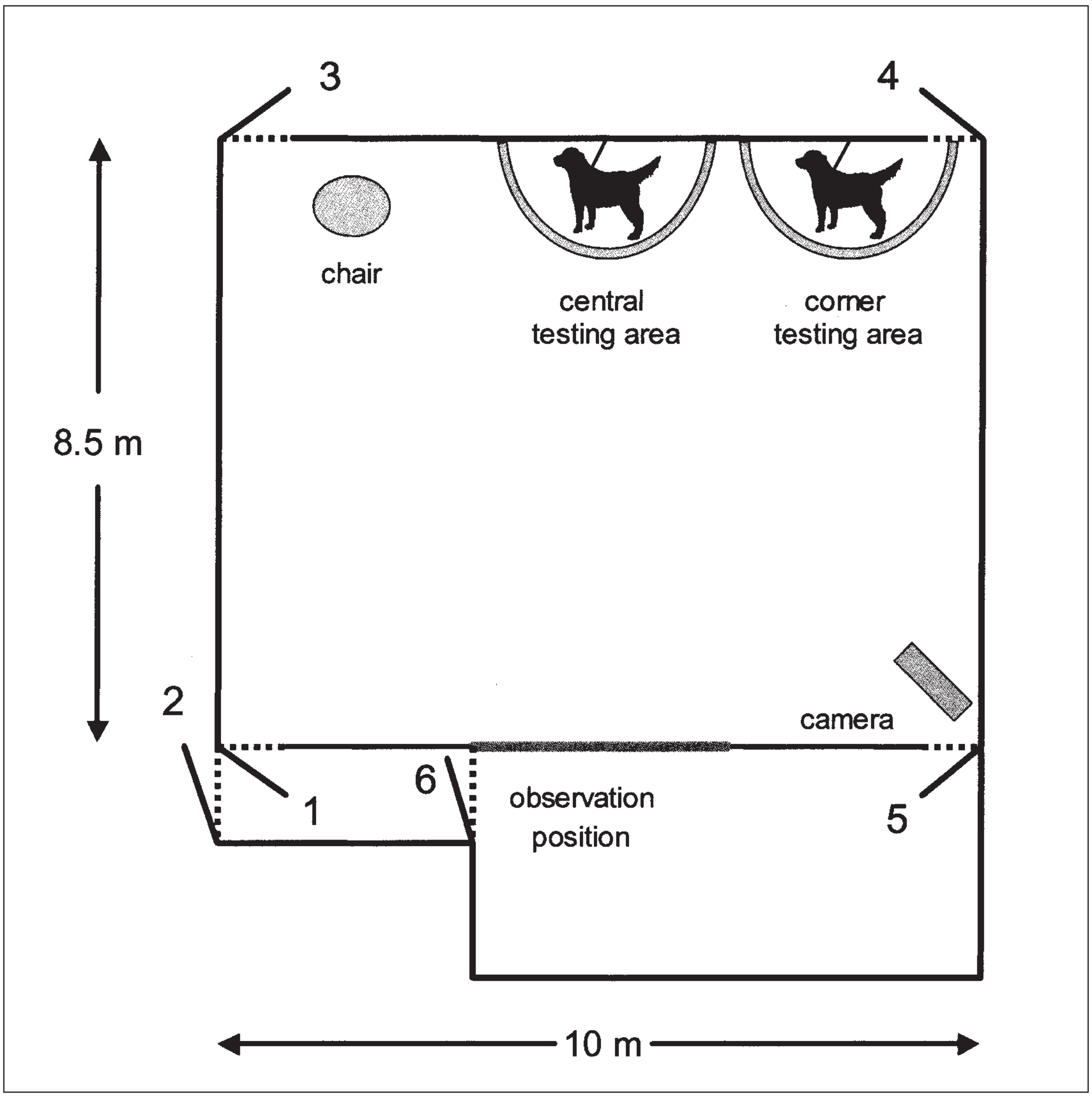

Fig. 1. Overview of the indoor testing facilities. Doors are numbered from 1 to 6. Subtests 6–12 and 17–22 were performed in the central testing area, whereas subtest 13–16 were performed in the corner testing area. If the owner was present, he/she would either sit on the chair adjacent to the central area or stand next to the dog in the corner testing area. The owner left and re-entered the room through door number 3. A description of the test can be found in the methods section.

University, which were previously described (Netto and Planta, 1997; Figure 1). Three persons performed the test: two test persons (one male and one female) and a camera man. If behavioral elements were unlikely to be visible or audible on the videotape, the test persons would always verbally report them. All subtests lasted 20 s, except for subtest 4 and 5. Pauses between the subtests were kept as short as possible. The 22 subtests are:

1. Two test-persons approach the dog-owner's car containing the dog, and both stare at the dog and knock on the car window. The owner is out of the dog's sight during this subtest.

After the first subtest, the owner walks the dog up and down with a leash outdoors and demonstrates the dog's obedience to the basic commands "sit," "down," and "come."

2. Confrontation with two free-running, barking stimulus dogs behind a fence (length 20 m). The owner walks the dog with a leash once along the fence and back again at a distance of 1 m from the fence. One of the test persons is also standing behind the fence.
3. Confrontation of the dog (in the absence of the owner) with a barking dominant stimulus dog behind a fence. A test person holds the golden on the leash. Again, one of the test persons is standing behind the fence.

After subtest 3 the dog is transferred to the adjacent test room (Figure 1), where all other subtests are carried out. The dog is given the opportunity to explore the test room before subtest 4.

4. The owner plays tug-of-war with the golden for 1 min using an unfamiliar toy. (O'Farrell, 1986; van der Wijk and Klasen, 1981)
5. A test person plays tug-of-war with the dog for 1 min using the same toy as in subtest 4. The owner is sitting on the chair in the test room.

The owner attaches the dog to a hook with a double leash in the central testing area.

6. The owner squeezes the skin on the dog's groins rather tightly.
7. Using an artificial hand, a test person pulls away the dog's feeding bowl while the dog is eating (dry dog food). The artificial hand is a plastic natural-looking model of a hand, with a stick attached to it. The stick is covered with a sleeve to hide the real hand of the test person. The bowl is pulled away and pushed back to its original position repetitively. At the start of this subtest, the owner places the bowl in the right position and then takes a seat on the chair next to the dog (van der Borg *et al.,* l991).
8. Using his/her own hand or an artificial one, the owner pulls away and pushes back the dog's feeding bowl while it is eating.

The owner now leaves the room through door 5, and subtests 9 through 12 are performed in the absence of the owner.

9. The male test person repeatedly opens an umbrella with an automatic opening device in front of the dog.
10. The female test person, dressed as a strange-looking woman walking with a stick, approaches the dog, tries to pet the dog using the artificial hand, and speaks in a strange high, piercing voice (Winkler, 1977).
11. The male test person claps his hands loudly in front of the dog.
12. The male test person threatens the dog by shouting and making hitting and kicking movements in the direction of the dog just out of reach of the dog (Wright, 1985).

The dog is moved to the corner of the testing room. Again, it is attached to a hook with a double leash by its owner. The owner is standing next to the dog during subtests 13–16.

13. Two persons surround and approach the dog quickly, while staring at the animal.
14. The male test person threatens the owner by yelling and shouting at him/her, and that test person pushes the owner with the artificial hand. The hand also moves in the direction of the dog several times (Beck *et al.,* 1975; Seiferle and Leonhardt, 1984).
15. Two persons corner dog and owner with two female stimulus dogs on the leash.
16. A test person with a dominant dog on the leash approaches the dog, stopping at a distance of 0.5 m from the edge of the corner testing area. The gender of the stimulus dog is the same as the gender of the golden retriever (Goddard and Beilharz, 1985).

The dog is transferred back to the central area.

17. A test person walks with the stimulus dog toward the owner (who is sitting on the chair), and the owner is asked to pet the stimulus dog and not to pay attention to his/her own dog (Goddard and Beilharz, 1985).

18. The dog is given its feeding bowl by its owner at a distance of 0.5 m from the same stimulus dog (Goddard and Beilharz, 1985).
19. The owner gives his/her dog's feeding bowl to the stimulus dog (Goddard and Beilharz, 1985).

The owner leaves the room again through door 3, so subtest 20 and 21 are performed in the absence of the owner.

20. A life-size doll (little girl), 65 cm tall, is taken at walking speed toward the dog by a test person. When reaching the dog, the test person tries to touch the dog with the doll's hand (Blackshaw, 1988; van der Borg *et al.,* 1991; Wright, 1985)
21. A test person wearing a dog mask approaches the dog.

The owner takes a seat on the chair again.

22. A test person pets the dog with the artificial hand.

These subtests are similar to subtests 1, 5, 6, 9, 11, 16, 17, 18, 21, 23, 27, 28, 31, 33, 34, 35, 36, 37, 38, 24, and 12 in the aggression test developed by Netto and Planta, except for some small practical alterations. For the sake of clarity, descriptive keywords will be added to subtest numbers in the remaining of this paper.

The following small deviations from the protocol occurred: the owner was present instead of absent during one of the subtests, the owner was standing instead of sitting during one or two subtests, the owner petted the dog for a short time at the start of one of the subtests, a test person instead of the owner gave the feeding bowl to the dog, and some of the subtests accidentally lasted more than 20 s (or 1 min for subtest 4 and 5).

Ethological and Statistical Analysis

All tests were recorded on videotape and subsequently analyzed with help of an ethogram (Tables I and II). Scoring was only performed during the 20 s or 1 min a subtest lasted, also when the subtest accidentally took more time. During this period, we scored how often the dog showed a certain behavior (continuous sampling), and these frequencies were added for the different subtests. Subtest 1, 2, 3, and 22 were not included in the sum because their standardization was moderate and the behavior of the dogs during these subtests was sometimes poorly visible on tape. The result of this ethological analysis was therefore a "behavioral profile" for each dog, consisting of the frequency of each of the behaviors listed in Tables I and II during subtests 4–21. Note that subtest 1, 2, 3, and 22 are not excluded from the general test results in the results section.

Table I. Ethogram of Aggressive Dog Behavior

Direct staring	The dog is staring at the stimulus. Often the pupils are slightly widened and the dog freezes.
Raising the hackles	Hairs on neck, back and hindquarters rise.
Stiff posture	Muscles in the body are tense; the dog looks stiff and does not move.
Barking	Short barking sound.
Growl-barking	Combination of growl and bark.
Growling	Low buzzing sound.
Baring the teeth	The dog pulls up its upper lip, so that its teeth are visible.
Pulling up the lip	Lips are pulled up slightly, but teeth are not visible.
Snapping	A snapping movement (mouth opens and closes, possibly accompanied by showing the teeth and/or growling and/or barking) associated with a short lunge forward (not maximally) or a quick head movement.
Attacking	The dog quickly moves forward maximally and makes snapping movements or actually bites (this may be impossible because of the subtest safety design), possibly accompanied by showing the teeth and/or growling and/or barking.

Three methods were applied for converting the behavioral profiles into a measure of behavioral phenotype:

1. First, only the most intense aggressive behaviors, snapping and attacking, were considered. A dog's phenotype could then be defined as the "snap/attack score": the total number of snaps and attacks recorded during subtests 4–21. Two-tailed Mann Whitney U tests were used to determine whether these scores corresponded to the owner-acknowledged classification. The nonparametric Mann Whitney U test was chosen to avoid assumptions about the underlying distribution of the variables.
2. It is a simplification to restrict the phenotypic measure to snapping and attacking behavior. Extension of the number of behavioral elements included in the phenotypic measure might make it more suitable for genetic analysis. Therefore the second definition of the phenotype that we used was the "total aggression score": the added frequency of snapping and attacking behavior and the threatening behaviors listed in Table I. These scores were again compared to the owner-acknowledged classification by means of Mann Whitney U tests.

Table II. Ethogram of Fearful Dog Behavior

Trembling	The dog is trembling all over its body.
Attempting to flee	The dog tries to increase the distance to the stimulus by moving forward or backward until the leash is stretched maximally.
Shrinking back	The dog shrinks backward or sideward, away from the stimulus, but it does not use the full length of the leash.
Seeking cover	The dog tries to hide behind its boss or something else with respect to the stimulus.
Support seeking	The dog approaches its owner, looks at its owner, and/or pushes itself against its owner, but it does not hide behind its owner.
Tongue flicking*	The tongue shortly appears from the front of the mouth.
Licking the beak*	The tongue shortly appears from the front of the mouth and licks the upper lip with a lateral movement.
Breaking eye contact	The dog obviously looks away from the stimulus for at least 3 seconds.
Lifting front paw	The dog lifts one front paw and keeps standing like this for a short time.
Smacking the lips	The dog opens and closes its mouth; this is no biting attempt and there is no movement forward.
Hunching	Hunching for a short time.
Startled movement	Short startled movement (no hunching) of the whole body.
Squeaking	High, squeaking sound.

*This behavior was not scored during subtests 7, 8, 18, and 19, because these subtests all involved food and the behavior was therefore considered to have no fear motivation.

3. Fear might play an important role in the etiology of canine aggression. Therefore the complete behavioral profiles of the dogs (including both aggressive and fearful behaviors) were subjected to principal components analysis (PCA). The aim of this third analysis was to reduce the large number of behavioral elements in the profiles to a small number of underlying variables ("components") based on patterns of correlations among the frequencies of occurrence of the behavioral elements. We might for example expect to find a component consisting of aggressive behaviors and a component consisting of fearful behaviors. Factor scores on the different components can then be used as a measure of the behavioral phenotype of the dogs. The behavioral elements "raising the hackles," "trembling," and "smacking the lips" were excluded from the PCA, because they occurred only incidentally. Scores on the behavioral elements "tongue flicking" and "licking the beak" were added, as were those on "seeking cover" and "support seeking." SPSS software was used for the PCA, components with eigenvalues over 1 were extracted, and the varimax method with Kaiser normalization was applied for rotation. Two-tailed Mann Whitney U tests were used to determine whether different owner-acknowledged classes had different median factor scores on the various components.

For the sake of clarity, the results of the questionnaires, the general test results, and the results of the three methods of analysis will each immediately be discussed in the results section.

Ethical Aspects

We obviously did not want our test to have any adverse effects on the future behavior of the dogs. Thus the test was aborted when it was too stressful for a dog and the owner could also terminate the test at any time. Dogs that did not complete the test were not included in this article. Because the majority of the test was performed inside the test room, chances were high that context learning occurred; that is, the dogs would associate the unpleasant experiences of the test with the test room and not with situations that they may encounter again in the future. The possibility of a future increase of aggressive behavior as a result of "winning experiences" during the test was minimized by trying to make sure that neither the stimulus dogs, nor the test persons would ever be "defeated" by the golden retriever.

RESULTS

Questionnaires

Based on the questionnaires, 24 dogs were classified as "owner-acknowledged nonaggressive," and 59 dogs were classified as "owner-acknowledged aggressive." The owner-acknowledged aggressive group included both dogs that had actually bitten their victims once or several times ("biters," $n = 44$) and dogs that had not bitten so far ("threateners," $n = 15$). This classification reflects the extent to which the owner regards his or her dog's aggressive behavior as a problem. Although different owners have different definitions of problem behavior (Hart and Hart, 1985; Mugford, 1984), we expect the degree of problems experienced by the owner to be highly correlated to the dog's tendency to behave aggressively.

The information provided by the owners showed that the aggressive behavior was not similar in all dogs.

Some dogs were only aggressive to people ($n = 20$), other dogs were exclusively aggressive to dogs ($n = 7$), and others were aggressive to both people and conspecifics ($n = 32$). Aggressive behavior to people was sometimes restricted to family members, whereas other dogs were aggressive to strangers, and the place where aggressive behavior usually occurred (within the dog's own territory or outside) also varied. Moreover, the severity of aggression varied: some dogs were responsible for several severe biting incidents, but others had only threatened so far. Environmental influences are probably involved in this phenotypic heterogeneity, but genetic variation may also play a role. It is possible that a clear genetic basis is only present in a subgroup of the dogs, and, although unlikely within a single breed, it is also possible that different gene mutations are responsible for different phenotypes. The key to success in this research project is therefore probably to find a homogeneous subgroup of owner acknowledged aggressive dogs in which aggression has the same genetic etiology.

General Test Results

All dogs performed the test according to the protocol. The majority (81 dogs) showed some of the aggressive behaviors listed in Table I during the test. Fearful behavior (Table II) was seen in all dogs during several subtests. The two most intense aggressive behaviors—attacking and snapping—were observed in only 29 golden retrievers (35%). This frequency is low compared to the 67% reported for the Netto and Planta dogs. The length of our test can probably explain this. In a pilot study, Netto and Planta showed that a test with more subtests elicits more aggressive behavior. We chose to abbreviate their test nevertheless, because Netto and Planta had a high number of "false positives" (owner-acknowledged nonaggressive dogs that attacked in the test). Another explanation is the breed of the subjects. All subjects in this study were golden retrievers, and they are probably less aggressive and more easily impressed than the "potentially aggressive breeds" that Netto and Planta mainly tested.

As expected from the Netto and Planta studies, the subtests varied in aggression-eliciting power. Ranking the subtests based on the mean number of snaps and attacks gave the following order: 14 - 2 - 12 - 19 - 22 - 1 - 8 - 10 - 9 - 11 - 20 - 6/7/21 - 3/15/16/18 - 4/5/13/17. Subtest 14 (threatening the owner) elicited the highest mean number of snaps and attacks (Figure 2a). Snapping and attacking never occurred during subtests 4 and 5 (both tug-of-war), 13 (cornering), or 17 (owner pets other dog). Netto and Planta expressed the aggression-eliciting power of subtests as the percentage of dogs that snapped or attacked during a subtest. This parameter gives the following descending order of subtests in the golden retrievers: 2/14/22 - 1/19 - 12 - 9 - 10 - 7/11/16 - 3/6/8/15/18/20/21 - 4/5/13/17. For the results presented by Netto and Planta, this order was: 19 - 18 - 14 - 17 - 15 - 16 - 12 - 1 - 3 - 11 - 13 - 6 - 20 - 10 - 9 - 7- 8 - 22 - 2. These orders differ substantially: subtest 17 (owner pets other dog), for example, gave rise to snap/attack behavior in many dogs in the Netto and Planta test, whereas this behavior was never recorded during our subtest 17.

In all subtests, threatening behavior was elicited in several dogs, and ranking them based on the mean frequency of threatening behavior resulted in the order: 21 - 19 - 17 - 2 - 14 - 1 - 12 - 11 - 20 - 22 - 9 - 10 - 3 - 18 - 4 - 5 - 16 - 13 - 15 - 8 - 7 - 6, with subtest 21 (dog mask) evoking the highest mean threatening frequency (Figure 2b). Aggressive behavior during subtest 4 and 5 (both tug-of-war) was always either barking, or growling, or both. This should probably be interpreted as play barking and/or play growling, because the dogs showed no threatening postures during these two subtests. Figure 2c shows that in all subtests fearful behavior was elicited in some dogs, and that subtest 9 (umbrella) gave rise to the highest mean fear frequency.

Snap/Attack Scores as a Measure of Behavioral Phenotype

A simple method of translating the test results into a measure of behavioral phenotype is to consider only the most intense aggressive behaviors: snapping and attacking. These are the behaviors that we were most interested in: they are the most hazardous to our society, and we would therefore like to unravel the genetic basis of these particular behaviors. For each dog a "snap/attack score" was calculated, which equaled the total number of snaps and attacks recorded during subtests 4–21. For molecular genetic analysis, the score may either be used as a quantitative measure of aggressiveness, or it may be used to subdivide the dogs into two or more phenotypic groups, for example, in a group with a snap/attack score of zero and a group with a score higher than zero. Sixty-two golden retrievers never snapped or attacked during subtests 4–21. Detailed information about the number of snaps and attacks performed by the animals is shown in Table III.

Owner-acknowledged aggressive dogs had significantly higher snap/attack scores than dogs that were

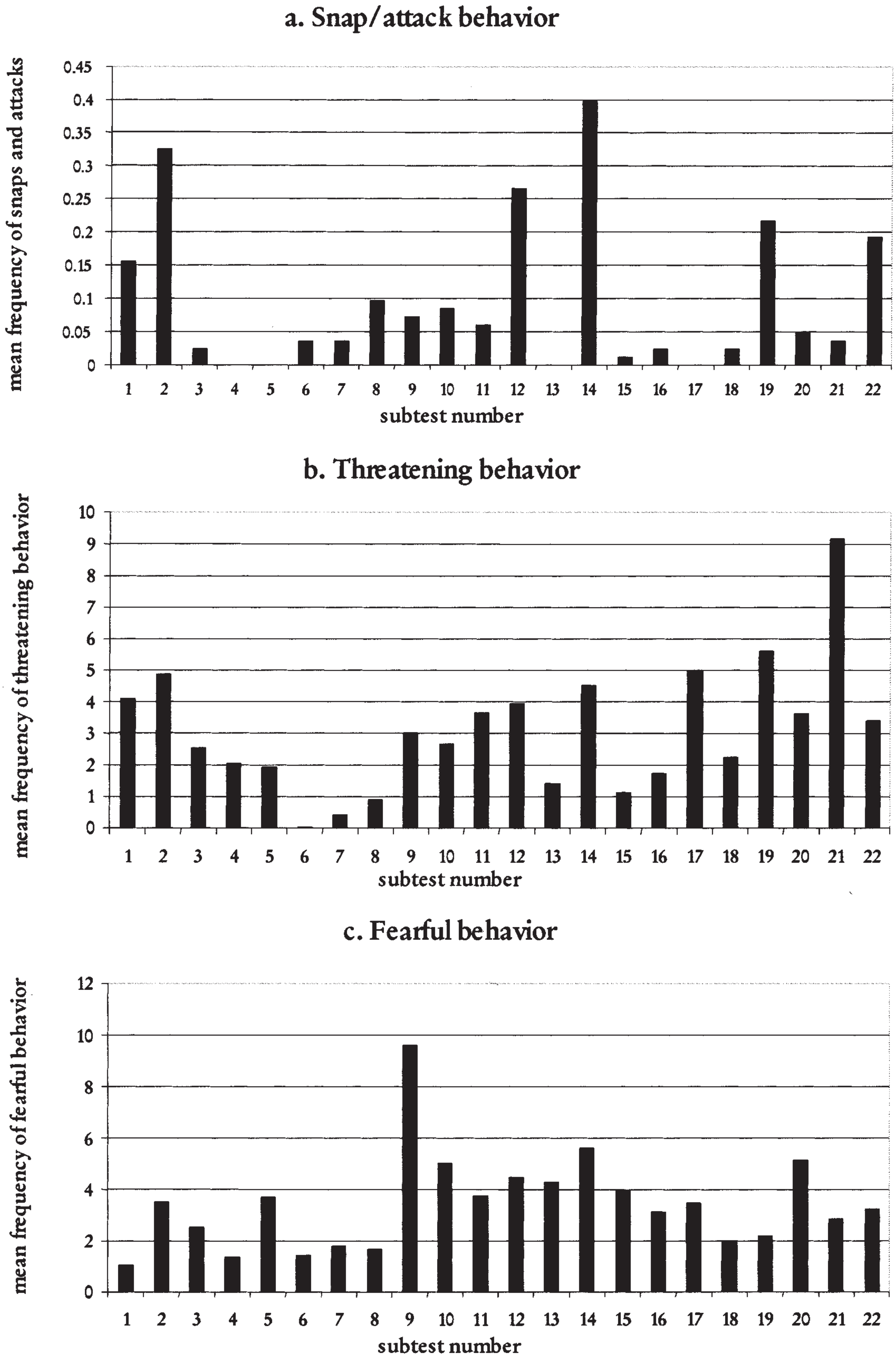

Fig. 2. Aggression- and fear-eliciting properties of the 22 different subtests. For each subtest, the mean frequency of snapping and attacking behavior of the dogs is depicted in Figure a, the mean frequency of threatening behaviors (i.e., direct staring, raising the hackles, stiff posture, barking, growl-barking, growling, baring the teeth, and/or pulling up the lip) is shown in Figure b, and the mean frequency of fearful behavior (i.e., trembling, attempting to flee, shrinking back, seeking cover/support, tongue flicking, licking the beak, breaking eye contact, lifting front paw, smacking the lips, hunching, startled movement, squeaking), is depicted in Figure c. Numbers on the horizontal axes correspond to the subtests described in the Methods section.

Table III. Phenotypes Expressed as Snap/Attack Scores for the Two Owner-Acknowledged Classes of Golden Retrievers

Number of attacks or snaps recorded during subtests 4–21	Number of owner-acknowledged nonaggressive dogs	Number of owner-acknowledged aggressive dogs
0	22	40
1	1	1
2	0	7
3	1	1
4	0	2
5	0	1
6	0	2
9	0	3
12	0	1
31	0	1

Note: Subtests 1, 2, 3, and 22 were not included in the frequencies.

nonaggressive according to their owners ($p = 0.018$). This significance relies mainly on the test results of the 24 owner-acknowledged nonaggressive dogs: only two of them snapped or attacked in the test. There was more discrepancy in the scores of owner-acknowledged aggressive dogs: 40 of them (68%) did not attack or snap. The following explanations can be given for this discrepancy:

1. A plausible explanation is the fact that the test does not include all possible aggression-eliciting stimuli and that it is mild because of its limited length. Exclusion of subtests 1, 2, 3, and 22 from the snap/attack scores lowered them, because, as can be seen in Figure 2a, subtests 1, 2, and 22 evoked a lot of snapping and attacking behavior. If subtests 1, 2, 3, and 22 were included in the snap/attack scores, only 54% of owner-acknowledged aggressive dogs had a snap/attack score of zero (compared to 68%) and it can therefore be concluded that the limited number of different stimulus situations presented to the dogs is one of the causes of the disagreement between snap/attack scores and owner-acknowledged classes.
2. The owner's story is unreliable in some cases: several owners are not skilled in observing dog behavior, and misinterpretations occur regularly.

The information provided by the owners revealed that the group of owner acknowledged aggressive dogs is heterogeneous with respect to several characteristics of the behavior. This provides additional explanations for the discrepancy mentioned above:

3. The target species varied (people, dogs, or both). When these three subgroups were analyzed individually, it became clear that only the dogs with a history of aggressive behavior toward both humans and conspecifics had significantly higher snap/attack scores than owner-acknowledged nonaggressive dogs ($p = 0.009$). Dogs that were reported to be aggressive toward humans only or toward dogs only were not significantly more likely to attack or snap during the test than owner-acknowledged nonaggressive dogs ($p = 0.116$ and $p = 0.444$, respectively). This will be discussed further in the section dealing with total aggression scores as a measure of behavioral phenotype.
4. Dogs that have never bitten strangers in the past (which often occurs in dominance aggression, Borchelt and Voith, 1982) would not be expected to bite the test persons. Therefore we used a χ^2 test to compare the familiarity of the victims (family members or strangers) between owner-acknowledged aggressive dogs that did not snap or attack during the test and owner-acknowledged aggressive dogs that did. There was no significant difference between the two groups ($p = 0.78$).
5. Dogs that only behave aggressively in their own territory are not expected to show aggression in the test. Therefore the place where the dogs had usually behaved aggressively (within their own territory, outside, or both) was compared between, on the one hand, owner-acknowledged aggressive dogs that did not snap or attack during the test, and, on the other hand, owner-acknowledged aggressive dogs that did. There was no significant difference between the groups ($p = 0.73$).
6. Threateners (dogs that had never actually bitten a person or another dog) were included in the owner-acknowledged aggressive group. This might partially explain the discrepancy because the snap/attack score only includes actual biting behavior. Therefore the analysis was repeated with biters only ($n = 44$). Sixty-six percent of the biters did not attack or snap in the test, so excluding the threateners would slightly improve the agreement between the owner's story and the test results.

Taking into account explanation 1, 3, and 6, we compared snap/attack scores for the complete test (including subtests 1, 2, 3, and 22) of owner-acknowledged nonaggressive dogs ($n = 24$) to those of dogs with a biting history toward both humans and conspecifics ($n = 26$). The aggressive dogs had significantly higher snap/attack scores, with a p-value of 0.000, and only 42% of them did not snap or attack during the test. In conclusion, the snap/attack score seems

Table IV. Phenotypes Expressed as Total Aggression Scores for the Two Owner-Acknowledged Classes of Golden Retrievers

Number of aggressive behaviors recorded during subtests 4–21	Number of owner-acknowledged nonaggressive dogs	Number of owner-acknowledged aggressive dogs
0–19	17	19
20–39	1	10
40–59	2	8
60–79	0	3
80–99	1	6
100–119	1	4
120–139	0	3
140–159	1	2
160–179	0	2
180–362	1	2

Note: Scores were grouped in classes. Note that the size of the last class (180–362) differs from the others. Subtests 1, 2, 3, and 22 were not included here.

to be a reasonable measure of aggressiveness for dogs with a biting history toward both humans and conspecifics. However, the overall usefulness of the score is questionable because of the low number of dogs showing snapping or attacking behavior.

Total Aggression Scores as a Measure of Behavioral Phenotype

An alternative to the snap/attack method is using the "total aggression score" (the total frequency of the aggressive behaviors listed in Table I during subtests 4–21) as a measure of the behavioral phenotype of the dogs. As was the case for the snap/attack scores, these total aggression scores can be treated both as a quantitative measure of aggressiveness and as a basis for subdivision of the dogs in phenotypic classes. The total aggression scores varied from 0 to 362 in the golden retrievers (Table IV).

Owner-acknowledged nonaggressive dogs had significantly lower total aggression scores than owner-acknowledged aggressive dogs ($p = 0.008$). The agreement between total aggression scores and owner-acknowledged classes was thus more significant than was the case for the snap/attack scores, but Table IV shows that some owner-acknowledged nonaggressive dogs had very high total aggression scores, and, on the other hand, several owner-acknowledged aggressive dogs had very low scores. Some of the explanations for this disagreement that were mentioned in the snap/attack section are also valid here, that is, the limited number of different stimuli presented in the test, the short duration of the test, and the moderate reliability of the owner's story. As was already mentioned, aggressive behavior during the tug-of-war subtests 4 and 5 should probably be interpreted as play barking and/or play growling. Therefore, total aggression scores were also calculated without these two subtests. These scores of owner-acknowledged nonaggressive dogs were again compared to those of owner-acknowledged aggressive dogs, but the resulting p values were not significantly different from those in which subtests 4 and 5 were included.

In the previous section, we demonstrated that there were substantial differences in the agreement between the owner's story and snap/attack scores for three subgroups of owner-acknowledged aggressive dogs (aggressive to only people, to only dogs, or to both people and dogs). This can also be observed for total aggression scores. Although dogs with a history of aggression toward only people had significantly higher total aggression scores than owner-acknowledged nonaggressive dogs ($p = 0.025$), the agreement was better for dogs with a history of aggression towards both people and conspecifics ($p = 0.010$). There was no significant difference between the total aggression scores of owner-acknowledged nonaggressive dogs and dogs with a history of aggression toward only dogs ($p = 0.595$). The explanation for this is probably that both snap/attack and total scores are a measure of overall aggressiveness: they are both the sum of frequencies observed during subtests where humans are the threatening stimuli ("people-subtests") and subtests in which conspecifics are the threatening stimuli ("dog-subtests"). Although dogs with a history of aggression toward exclusively humans might often show aggression during people-subtests, their total aggression score does not necessarily have to be high because this score also includes behavior during dog-subtests. The reverse is true for the dogs with a history of aggression toward exclusively conspecifics, and it is even worse in their case, because the number of dog-subtests is much smaller than the number of people-subtests. Addition of data from different subtests therefore seems to be an inadequate method of phenotyping for these two groups of dogs. It is in their case probably more effective to separately analyze the behavior during classes of subtests with similar stimuli.

In conclusion, total aggression scores are more useful than snap/attack scores, because the former agree better with the owner's story. In addition, the higher variation in the total aggression score also suggests that it is a more realistic measure of aggression than the snap/attack score.

Principal Components Analysis of Aggressive and Fearful Behavior

In the third and most extensive method of converting the behavioral profiles into measures of phenotype, a principal components analysis (PCA) was applied on the complete behavioral profiles of the dogs. PCA attempts to identify underlying variables (components) that explain the pattern of correlations within the frequencies of behavioral elements. The factor scores on the different components of the PCA solution can be used to classify the dogs. As was the case for snap/attack and total aggression scores, these factor scores can be treated both as a quantitative measure of aggression and/or fear, and as a basis for subdivision of the dogs in phenotypic classes. Six components, which explained 66% of the total variance between the dogs, were extracted from the data. Factor loadings were generally moderate to high and there was little cross loading between the components. The rotated component matrix is shown in Table V. Behavioral elements that contributed to the formation of the first component were mainly threatening (although "snapping" also had a factor loading of 0.528 on this component). More severe aggressive behavioral elements such as attacking and snapping had the highest factor loadings on the second component. The other four components all consisted of fearful behavioral elements, with an emphasis on active behaviors like "attempting to flee" in the third component, on startle in the fourth component, on support seeking in the fifth component, and on uncertainty in the sixth component.

Factor scores are only expected to differ between owner-acknowledged groups for the first two components, because the owner-acknowledged classification is based on aggressive behavior and not on fearful behavior. As expected, owner-acknowledged aggressive dogs had significantly higher factor scores on component 1 ("threatening") than owner-acknowledged non-aggressive dogs ($p = 0.040$). The difference between factor scores on component 2 did not reach significance ($p = 0.065$), but a trend was clearly visible. Factor scores on the other four components did not differ significantly between these owner-acknowledged groups, as was expected ($p = 0.34$, $p = 0.26$, $p = 0.25$, and $p = 0.85$ for, respectively, components 3, 4, 5, and 6).

Table V. Rotated Component Matrix Resulting from Principal Components Analysis of the Behavioral Profiles of the Dogs

	Component					
	1: threatening (22.9%)	2: attacking (13.4%)	3: active fear (9.28%)	4: startle (7.36%)	5: support seeking (6.79%)	6: uncertainty (6.22%)
Stiff posture	0.796					
Direct staring	0.876					
Growling	0.715					
Pulling up the lip	0.739					
Attacking		0.878				
Baring the teeth		0.940				
Barking		0.745				−0.341
Snapping	0.528	0.558				
Attempting to flee			0.828			
Shrinking back			0.546	0.311		−0.418
Seeking cover/support			0.492		0.597	
Hunching			0.668			
Startled movement				0.684		
Lifting front paw				0.608		
Growl-barking				−0.506		
Squeaking					0.731	
Tongue flicking						
Licking the lips						0.770
Breaking eye contact						

Note: The names of the components are based on the behavioral elements that contributed most to them. Percentages between brackets represent the percentage of variance explained by the component. Factor loadings between –0.3 and 0.3 are not presented in the table.

Interestingly, factor scores on component 1 did not differ significantly between owner-acknowledged nonaggressive dogs and the subgroups of owner-acknowledged aggressive dogs (aggressive to only people, aggressive to only dogs, or aggressive to both, $p = 0.195$, $p = 0.139$, and $p = 0.053$, respectively).

The results of this PCA have to be treated with caution. A drawback of PCA is that it is often hard to test the reliability of the solution (Tabachnick and Fidell, 2001). Splitting the group of dogs in two random halves and then repeating the PCA is an appropriate method to test this reliability, but in this study, the sample size in such split groups is too small for a reliable PCA: correlation coefficients tend to be less reliable when estimated from small samples (Tabachnick and Fidell, 2001). It would therefore be interesting to repeat the analysis with a higher number of dogs in the future. Until then, we must regard the use of the factor scores as a promising but not completely reliable method of phenotyping the dogs.

We also performed a principal components analysis on aggressive behaviors, fearful behaviors, and postures. Postures are important in communication between dogs, and the posture of a dog while behaving aggressively is an important indication of the motivation for the behavior. The position of the ears, head, and tail of the dog were used to determine the posture. However, this did not improve the solution of the PCA (results not shown).

DISCUSSION

Standardization

It is extremely important that the test conditions are standardized because aggression is such a complex trait (van der Staay and Steckler, 2002). However, it is hard to standardize behavioral tests like the one described in this paper. The first three subtests were performed outside, so environmental variation was unavoidable (e.g., weather conditions, sounds, etc.). We therefore decided to exclude these subtests from the analyses, but the dogs were nevertheless presented with slightly different situations before subtest 4 (tug-of-war), which may have caused variation in their test performance. There was also variation in the environment of the test room: air temperature, sounds coming from outside, etc. Moreover, variation was introduced by the owners, who would not always comply with our protocol (e.g., standing instead of sitting), and by the test persons (individual variation in precise execution of the subtests, exact time the subtests consumed, etc.). Scoring is also subject to considerable variation.

Two sources of variation could be prevented in the future. First, we used test persons that were aware of the history of the dogs, and they may have approached the aggressive dogs differently. It is preferable to use test persons unfamiliar with the dogs in the future. Secondly, not all subtests were consistently performed by test persons of the same gender. Subtests 9 (umbrella), 11 (clapping), 12 (hitting), and 14 (threatening the owner) were always performed by the male test person and subtest 10 (strange woman) was always performed by the female test person, but subtests 3 (dominant dog behind fence), 5 (tug-of-war), 7 (pulling away feeding bowl), 16 (dominant dog), 20 (doll), 21 (dog mask), and 22 (petting) were performed by the male test person for some dogs, and by the female for other dogs. Although the test person is not the threatening stimulus in these subtests, it is better to standardize the gender of the test person for all subtests, because dogs are known to respond differently to males than to females (Lore and Eisenberg, 1986; Wells and Hepper, 1999).

Evaluation of the Approach of Addition of the Subtests

The three methods of analysis presented in this paper were all applied on a dataset in which frequencies were added for the different subtests. Classifications of aggressive behavior in animals are often based on the nature of stimuli eliciting the behavior. This implies that we have added scores on different subtypes of aggression. That approach is based on the hypothesis that the golden retrievers have a genetically based lower threshold for aggressive behavior under various circumstances. This might be one of the manifestations of a lack of impulse control. Impulsivity has been studied in various animals, and it is believed to be under control of the serotonergic system (Coccaro *et al.,* 1997; Feldman *et al.,* 1997; Peremans *et al.,* 2003; Soubrié and Bizot, 1990). It is therefore possible that the aggressive behavior in the golden retrievers is caused by a mutation in one of the genes of the serotonergic system. An interesting plan for the future is to study impulsivity in more detail by measuring the time passing between the presentation of a stimulus and the dog's reaction. This "waiting time" is a measure of impulsivity (Soubrié and Bizot, 1990).

Only one tool is available for evaluating the approach of addition of the subtests: a comparison with the owner's story. This owner-provided information will not be accurate in some cases, and we therefore expected a certain discrepancy between owner-acknowledged classification and test results. Moreover, Mann Whitney U tests were used for all comparisons and these tests are normally used to compare two independent groups. The owner-acknowledged aggressive and nonaggressive groups are not independent: most of the nonaggressive dogs are siblings of a number of aggressive dogs. Siblings have experienced a common early environment, and they are genetically more similar than unrelated dogs. Therefore they are expected to behave more similarly than unrelated animals, and this will make it more difficult to find a significant difference in test results of the two groups. Keeping this in mind, the approach of addition of the subtests has given very decent results: snap/attack scores, total aggression scores, and factor scores on the two aggressive components in the PCA solution were significantly higher in owner-acknowledged aggressive dogs. However, the questionnaires revealed that the group of owner-acknowledged aggressive dogs was heterogeneous, and the agreement between test results and the owner's story was highest for the dogs with a history of aggression toward both people and conspecifics. This implies that the approach of addition subtests is most adequate for this subgroup of owner-acknowledged aggressive dogs, and maybe only these dogs do have a lowered overall aggression threshold. An attractive idea is to use both test results and information provided by the owners for selecting a small, homogeneous group of aggressive dogs for genetic analysis. For example, we could initially pick out the dogs that are aggressive to both people and conspecifics according to their owner and consequently further reduce the study group to dogs that had high total aggression scores in the test.

It will be very interesting to see what results emerge from the second approach, in which classes of subtests with similar stimulus situations will be analyzed separately. As was already explained in the introduction to this paper, the hypothesis underlying this approach is that different genetic mechanisms control different subtypes of aggressive behavior. Naumenko *et al.* (1989) presented evidence for this hypothesis when they discovered that selection of Norway rats for reduced fear-induced aggression toward people resulted in a decrease in irritable aggression, but no change in intermale and predatory aggression. Not much is known about the genetic basis of subtypes of canine aggression. It is claimed that fighting dogs have been selected exclusively for aggression toward dogs (Lockwood and Rindy, 1987), but there is no scientific evidence that this selection has not simultaneously increased aggressive behavior toward people.

One-Zero Sampling

Although the results presented in this paper were based on a continuous sampling method, we also performed all calculations with application of one-zero sampling (in which it was only noted whether the dog showed the behavior at least once or not at all during a particular subtest). Here, the agreement between the owner's story and snap/attack or total aggression scores was either identical or slightly lower than for continuous sampling, and the principal components analysis did not give clear results. Therefore it was not considered useful to present these data here.

CONCLUSION

The total aggression scores method was the best of the three methods that were presented in this paper because it showed the best agreement with the owner's story and the highest variation in the study group. However, the results of the principal components analysis were also promising. The approach of addition of the behavioral frequencies observed during different subtests worked best for dogs that were owner-acknowledged aggressive to both people and dogs. This implicates that only in these dogs a lowered "overall aggression threshold" is possibly present. This finding confirms our suspicions that the group of aggressive golden retrievers studied here is heterogeneous. Analysis of classes of subtests separately will increase the insight into the etiology of the aggressive behavior in the other dogs. However, it remains to be seen whether the level of standardization of the test meets the high requirements of molecular genetic studies on complex traits. The results from genetic studies on the golden retrievers will reveal this in the future.

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