Habituation responses in wild reindeer exposed to recreational activities

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Abstract: Displacement is the effect most often predicted when recreational activities in wild reindeer (*Rangifer tarandus tarandus*) areas are discussed. Wild reindeer in Blefjell (225 km^2) are exposed to humans more frequently than in Hardangervidda (8200 km^2), from which the Blefjell herd originate. We recorded fright and flight response distances of groups of reindeer in both herds to a person directly approaching them on foot or skis during winter, summer, and autumn post-hunting and rutting season in 2004-2006. The response distances sight, alert, flight initiation and escape were shorter in Blefjell than in Hardangervidda while the probability of assessing the observer before flight tended to be greater in Blefjell. To test whether these results could be due to habituation or genetic influence of semi-domestic reindeer previously released in the Blefjell region, we compared the genetic variation of the Blefjell reindeer with previously reported variation in semi-domestic reindeer and in the wild reindeer from Hardangervidda. Microsatellite analyses revealed closer genetic ancestry of the Blefjell reindeer to the wild Hardangervidda reindeer and not to the semi-domestic reindeer at both the herd and the individual level. We conclude that the decreased flight responses in Blefjell reindeer appear to be a habituation response to frequent human encounters rather than traits inherited from a semi-domestic origin.

Key words: fright responses, genetic variation, habituation, microsatellites, tourism, wild reindeer.

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Introduction

With increasing urbanization, ecotourism and off-road recreation including hunting, opportunities for confronting wildlife in an inadvertently threatening manner as well as intentional harassment appear inevitable. Human-wildlife interactions may result in a variety of effects including short-term flight behavioural patterns, habitat avoidance and/or activity alterations with energetic implications affecting reproduction and mortality (see review by Stankowich (2008)). It has long been recognized that learning plays an important role in the manner and degree to which ungulates respond to humans (Geist, 1971). There are three major learned responses of wildlife towards recreationists: habituation, attraction and avoidance (Knight & Cole, 1995), and all of these apply to reindeer. Avoidance is the mainstream effect most often predicted when recreational activities in wild reindeer areas are discussed (e.g. Vistnes 2008). Nevertheless, animals including reindeer show decreased flight responses in areas with relatively high amounts of human activities (Colman *et al.*, 2001; Stankowich &

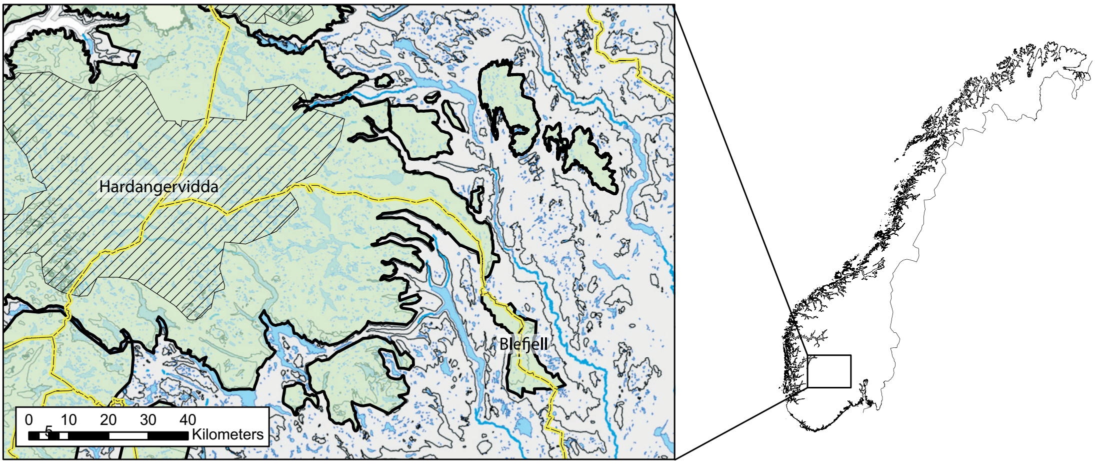

Fig. 1. The location of the study areas Hardangervidda and Blefjell delineated by the 1000 m contour line. The hatched area illustrates Hardangervidda national park.

Blumstein, 2005; Reimers *et al.*, 2009) and (E. Reimers *et al.*, in prep.), indicating the ability to habituate towards human activities..

Flight initiation distance is the distance at which an animal begins to flee from an approaching predator (Ydenberg & Dill, 1986). Because it is easy to measure and correlates with other key aspects of escape behaviour, e.g. alert distance (Blumstein *et al.*, 2005), it is an excellent and widely used metric with which to measure animal fearfulness. Habituation and former experience with predators significantly influences an ungulate's perception of fear. In their review, Stankowich & Blumstein (2005) found that populations with few predators flushed at greater distances than those where predators were common. All predator studies in their review classified humans as the predator and measured differences in flight initiation distance between populations that differed in human density. If wild ungulate populations exposed to relatively high amounts of human activities have become habituated to humans in a non-threatening context, they are likely to perceive less risk when approached by humans than would animals in populations where encounters with humans are rare (Colman *et al.*, 2001; Lund, 2008).

Among the 23 wild reindeer herds in southern Norway, we classify three types of reindeer on basis of their origin (Reimers & Colman, 2006): (1) the original wild reindeer with minor influence and genetic mixing from previous semi-domestic reindeer herding activities, (2) wild reindeer with some influx of animals from past semi-domestic reindeer herding units operating locally in the same mountain areas (e.g. Hardangervidda and Blefjell) and (3) feral reindeer with a semi-domesticated origin (reindeer released or escaped from past reindeer husbandry units).

Although reindeer are considered to be at an early phase of domestication (Baskin & Skogland, 2001), semi-domestic reindeer generally exhibit more relaxed fright and flight behaviour compared to wild reindeer (Reimers *et al.*, 2000; Reimers *et al.*, 2006). Therefore, when comparing fright or flight behaviour between reindeer herds, it is important to know the origin and complete history of the herds in question.

In the present work we address the question of habituation in wild reindeer comparing fright and flight behaviour in two herds, Hardangervidda and Blefjell, differing in their daily amounts of exposure towards human activities. Blefjell is a neighbouring spit area to Hardangervidda and has a much higher level of human activities and reindeer-human encounters compared to Hardangervidda. The present Blefjell reindeer herd originate from immigrating Hardangervidda reindeer and possibly also from semi-domesticated reindeer introduced in 1961. To control for possible confounding effects from the previous introduction of semi-domestic reindeer, we compare the genetic variation in fourteen microsatellites in the Blefjell reindeer with previous reported variation in three semi-domestic reindeer herds and in three herds of wild reindeer from the Hardangervidda region.

Study area and study herds

Blefjell and Hardangervidda are alpine areas located in southern Norway (60°25´N; 9°15´E) and cover 225 km^2 and 8200 km^2 respectively (Fig. 1). Forty-two percent of the Hardangervidda mountain habitat (3422 km^2) is located within a national park, and most of the area is more remote and less accessible than the neighbouring spit area Blefjell.

The Norwegian Tourist Association (DNT) run 13 tourist resorts distributed in the central parts of Hardangervidda. Three of these resorts are operated with service staff and kept open during summer and during Easter. The remaining 10 are self service cabins which may be utilized by the public the whole year. During the period 1990 to 2006 the average annual number of over night visitor in the 13 tourist resorts has been stable at 5700 persons in winter and 14 000 in summer (DNT Oslo branch, pers. comm.).

With short distances to the major settlements in the eastern part of southern Norway, Blefjell has marked itself as a very attractive area for recreational activities for the approximately 1.5 million people inhabiting areas within 100 km from Blefjell. As a result, areas close to the timberline are heavily utilized in terms of cabins and tourist resorts with an extensive recreational use of the alpine and the below timberline areas. According to Garås (2004), the number of people visiting Blefjell in the holiday seasons is estimated to more than 19 000 per day similar to the total number of over night visitors at the Hardangervidda tourist resorts during the whole year. The most recent registration (Lurås & Flaget, 2006) and E. Lurås (pers. comm.) shows ca. 3500 cabins and 8 camping sites with a total of 450 permanent camping trailers.

The Hardangervidda reindeer herd has fluctuated considerably over the last 50 years. Rapid herd increase to possibly 20-30 000 animals during the 1960s was followed by a dramatic herd decline as a result of reduction harvest. After a hunting ban in 1971-72, the herd increased more rapidly than anticipated by the management authorities and reached an all time high in 1983 of some 30 000 animals. At this stage, some groups immigrated into neighbouring areas (Nordfjella and Setesdal-Ryfylkle) including the spit area Blefjell. A large hunting quota that year (16 000 animals) and the following years brought the herd down to an all-time low of less than 5000 animals in 2001 (Lund, 2001). Restricted hunting in the following years resulted in a slow and apparently controlled herd increase that left the herd at 6000 to 8000 animals in our study period 2004-06.

Historically, wild reindeer migrated between Hardangervidda and Blefjell, as indicated by pitfall systems from the period 1400-1500 (Bakke, 1984) and public observations until extensive hydroelectric development, human infrastructure and herd size reduction in Hardangervidda eventually reduced or closed the migration flow. Presently, roads, infrastructure and hydroelectric dams (Sønstevatn, Kalhovdfjorden, and Mårvatn) limit reindeer migration between the two study areas. Small groups of wild reindeer from Hardangervidda, primarily males, were occasionally seen in winters in Blefjell until 1960. Apparently, these visiting male groups moved back to Hardangervidda during springtime. In 1961, 30 reindeer (23 females and 7 males) bought from a neighbouring semi-domestic reindeer herd (Ål and Hol tamreinlag) were released in the area in an attempt to build up a local Blefjell wild reindeer herd (Garås, 2004). A few males were killed the following year in Hardangervidda and the rest apparently disappeared. In 1968 and in 1970 groups of visiting wild reindeer from Hardangervidda (150 to 200 animals) were observed in Blefjell and in winter 1971 some 30 reindeer remained in Blefjell and gave rise to a local resident herd that increased to 60 animals including 10 calves in 1974 and 75 animals including 12 calves in 1975. The herd continued to increase and an air survey in March 1984 showed 276 animals. Herd size in the study period is estimated at 120 animals (Lurås & Flaget, 2006).

Both herds are hunted. In Hardangervidda 2683 animals were killed in 2000, dropping to 2144 animals in 2001, 1417 animals in 2002, 133 animals in 2003 and 18 animals in 2004. The corresponding annual harvest in Blefjell varied between 13 and 19 animals in the same period. Hunting appears to be the only important mortality factor as wolf (*Canis lupus*) is essentially absent from the areas and wolverine (*Gulo gulo*), golden eagle (*Aquila chrysaësetos*), and lynx (*Lynx lynx*), although permanently present or present as stranglers, exert minor predatory influence.

Methods

Fright and flight data

Fright- and flight data were collected in Blefjell in 2005 and 2006 and in Hardangervidda 2004 to 2006. During 3 sampling periods of March-April (winter), July-August (summer), and October (autumn; after hunting and during the rutting season) one observer on foot or on skis and dressed in dark hiking clothing approached reindeer directly during daylight hours. The observer measured response distances between the reindeer and the observer and the resultant displacement distance by the reindeer after taking flight using laser binoculars and mononoculars (Leica Geovid 7×42 BDA or Leica Rangemaster 1200 Scan; 1-m accuracy at 1000-1200 m).

We used wildlife response distance terminology and methodology recommended by Taylor & Knight (2003) with the modifications following Reimers *et al.* (2009): 1) Encounter distance (synonymous with start distance) was distance between the observer and the closest animal in a group of reindeer before the start of an approach. 2) Sight distance was distance between the observer and the closest animal when animals in the group displayed an alerted behaviour directed at the observer. 3) Alert distance was distance at which the reindeer group exhibited an increased alert response by grouping together or individuals urinating with one hind leg extended outward at an exaggerated angle, while staring at the directly approaching observer. 4) Flight initiation distance was distance from the approaching observer to the group when the reindeer initially took flight. 5) Escape distance (synonymous with distance moved) was shortest straight-line distance from where the reindeer took flight in response to the observer to where the reindeer resumed grazing or bedded down. 6) Assessment time was time elapsed from alert to flight initiation estimated from measured distances and assuming a constant observer speed of 4 km/hr. We then divided assessment time into 2 classes to estimate assessment probability: > 1 second, which we classified as animals assessing the observer, and ≤ 1 second, which we classified as animals not assessing observer.

Upon locating a group of ≥ 2 animals, we recorded 6 additional parameters: sample month(s), group size (i.e., small: < 20 animals, medium: 20 to 50 animals, and large: > 50 animals), group composition (i.e., mixed, all ages and both sexes; males, yearlings, and older; females and calves), dominant activity of the group when first sighted (i.e. lying or grazing), wind direction relative to the observer (i.e., tail wind or into the wind including crossways to the wind), and topography (terrain ruggedness) of the surrounding area (i.e., level or rugged).

When a group of reindeer was first sighted, the observer measured encounter distance, then approached the center of the group directly at a constant speed of 4 km/hr with ≤ 6-sec stops to measure sight distance, alert distance and flight initiation distance. The observer continued until reaching the position where the reindeer were located at the start of the approach. We made all measurements from the position of the observer to the closest animal in the group. The same group of reindeer was sometimes approached two or more times during the same day. Therefore, of 85 total encounters, we measured 11 approaches on the same group on the same day. Repeated approaches were separated by ≥ 1 hour between the first and second approach and 2 hours between the second and subsequent encounters.

In Hardangervidda, most of the reindeer are found in few large mixed groups during the rut. During winter most males leave the mixed groups and regroup in medium size groups (50 to 100 animals or larger (100 to 200 animals) while females, calves, young males and a few older males remain in larger groups (> 100 animals). During summer, the reindeer are distributed in a variety of group sizes from less than 100 to large groups up to 3000 and more animals. As the number of reindeer groups available for disturbance testing is few in Blefjell, the same group was frequently disturbed several times during every season and sometimes during the same day. We differentiated between groups on basis of number of animals and date, time and location of the group and in some situations animal caracteristics.

Genetic data

We obtained 24 tissue samples from Blefjell during the regular hunt in 2006-2007. DNA was isolated using DNeasy kit (QIAGEN), following the manufacturer's guidelines. All samples were analysed for 14 reindeer-specific microsatellites (NVHRT-01, NVHRT-03, NVHRT-16, NVHRT-21, NVHRT-24, NVHRT-31, NVHRT-48, NVHRT-73, NVHRT-76 (Roed & Midthjell, 1998) and RT-1, RT-5, RT-6, RT-9, RT-27 (Wilson *et al.*, 1997). The amplification was performed on a GeneAmp PCR System 9700 (Applied Biosystems) as previously described [see methods in Røed *et al.* (2002)]. PCR products were electrophoresed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Use of these markers in a previous study has given evidence of low scoring errors (< 5 %) due to stutter bands, allelic dropout or null alleles (Roed *et al.*, 2008).

The genetic variation was compared with previous reported variation in three herds of semi-domestic reindeer and three herds of wild reindeer from Southern Norway (Roed *et al.*, 2008). The reference herds of wild reindeer (Nordfjella, Setesdal/Ryfylke, and Hardangervidda) represent the three main herds within the Hardangervidda region from which eventually wild reindeer in Blefjell originate. The three reference herds of semi-domestic reindeer from southern Norway (Filefjell, Vågå, and Røros) are assumed to represent an eventual ancestry to semi-domestic reindeer.

Statistical methods fright and flight data

Response distances were transformed into their natural logarithms prior to analysis. Our aim was to identify if frequent exposure to human infrastructure have changed the response distances and if other biologically relevant variables were influential. Encounter distance was not different in the two areas (Table 1), and thus did not imbalance the comparison.

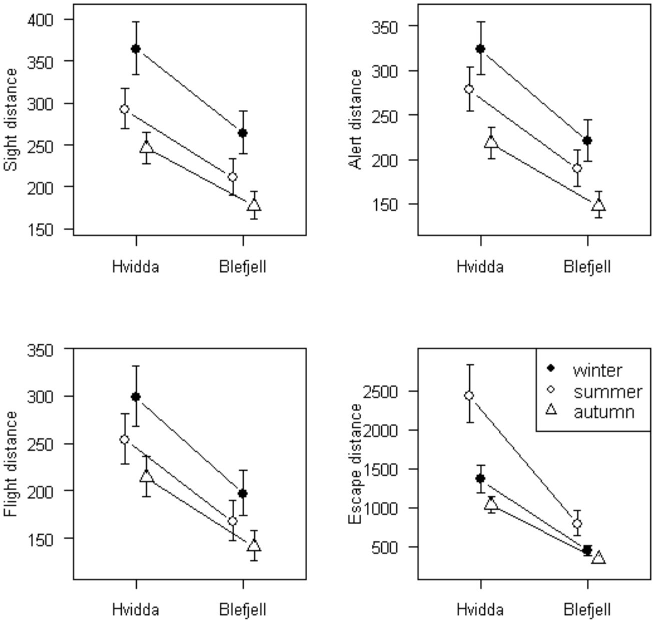

Fig. 2. Predicted values (m) of a) sight distance, b) alert distance, c) flight initiation distance and c) escape distance of reindeer groups disturbed by an intruding person in Hardangervidda and Blefjell, south central Norway. Reference levels of categorical variables (if included in the model; see Tables 2-5) are rugged terrain and mixed group structure. For the numerical variable encounter distance the predictions are based on the mean. Error bars are 1 SE.

Reindeer groups were repeatedly approached on the same day. Due to this we chose to analyze the data with linear mixed-effects models (LME) (Pinheiro & Bates, 2000) and generalized linear mixed-effects models (GLMM) (Woods, 2006) when the response variables were respectively normally and binomially distributed. Reindeer group was included a priori as a random intercept in all models and was not subjected to model simplification. The consecutive number of approach on a group per day (1 for the first approach, two for the second approach etc.) was included as a numerical fixed effect in the full models (to check for potential habituation or sensitization towards the disturbances), but subjected to model simplification as detailed below.

Sight, alert, flight initiation and escape distances were analyzed with LME. For hypothesis testing of fixed-effects terms in LMEs, Pinheiro & Bates (2000) recommend to use marginal F-tests. Following the backwards model selection philosophy of Crawley (2005), we started with full models containing all biologically plausible main effects and two-way interactions. The full LME models contained as fixed effects: area, season, encounter distance (ln transformed and centered at the mean), group size, group composition, wind direction relative to the observer, terrain type, activity type, the numbered approach per day (centered at one), and the two-way interactions hunting area x season, group size x group composition, area x encounter distance, season x encounter distance and wind direction x terrain type. We ran marginal F-tests on the full models, removed the fixed-effect variable with the highest *P*-value and repeated this procedure until only significant variables were retained. The exception was the main effect of area, which we retained in the models irrespective of significance as it is our variable of primary interest. LME models were fitted using the library nlme (Pinheiro & Bates, 2000) implemented in the statistical software R (R Development Core Team 2008). Distributions of residuals were checked with diagnostic plots to check for any strong deviation from normality.

The probability of assessing an observer before taking flight was analyzed with a GLMM, using the function lmer in the R library lme4. The binomial response variable in this model takes a value of 1 if assessment time >1 s and 0 if assessment time ≤1 s (immediate flight after alert). Small sample size prevented an assessment model that included all the variables tested in the full LME models. The initial and final model included area and season. Reindeer group was included a priori as a random intercept.

To facilitate interpretation of the effect of area on the five vigilance measures, we plotted predicted values for each season and area (Figs. 1 and 2). The predict function for LME models does not provide estimates of standard error and there is no predict function developed for GLMMs in R. As the random effect was small compared with the residual error, we used the corresponding (i.e., the fixed effects from Table 2-5) linear models (LM; for sight, alert, flight, and escape distances) and generalized linear model (GLM; probability of assessing) for plotting purposes.

We also present predicted values at a normal scale (in meters and percentages) in text in the result section. These values are predicted values from the models in Tables 2-6 and obtained by varying only the factor of interest while keeping all other factors constant at a reference level. As reference levels we used area Blefjell, season summer, mean encounter distance, mixed group structure (the level after the *vs.* term, such as “Blefjell” for area and “summer” for season) and rugged terrain. All terms are not included in all models (Table 2-6).

Statistical analyses of genetic data

Random mating within populations was assessed by exact tests of Hardy-Weinberg equilibrium across the 14 microsatellite loci using GENEPOP 3.4 with default settings (Raymond & Rousset, 1995). The amount of genetic variation is expressed as mean number of alleles, allele richness and gene diversity (Nei, 1987) in each herd across loci using FSTAT 2.9.3 (Goudet, 2001).This program was also used to assess the genetic structure among herds (F_{ST}).). Significance levels for HWE and F_{ST} tests were corrected for Type 1 and Type 2 errors according to the False Discovery Rate procedure (Benjamini & Hochberg, 1995).

Genetic distances D_A (Nei *et al.*, 1983) among the herds were calculated and a neighbour joining tree built with 1000 bootstraps on loci using POPULATIONS (available at http://www.pge.cnrs-gif.fr/bioinfo/populations/index.php). The tree was visualised using TREEVIEW1.6.6 (Page: 1996).

Genetic structure at an individual level was analysed by the Bayesian assignment approach as implemented in the software STRUCTURE (Pritchard *et al.*, 2000). The log likelihood of our data [ln Pr(X | K)] was estimated given different numbers of genetic clusters ($K \in [1,6]$, using an admixture model with uniform priors ($\alpha=1$, $\alpha_{max}=50$), correlated allele frequencies, 50 000 burnin cycles and 500 000 MCMC iterations. All analyses were run without prior herd information, and were repeated 10 times for each K value.

Results

Fright and flight behaviour

We made 20 approaches in Blefjell (2005-2006; winter: 7, summer: 8, autumn: 5) and 65 approaches in Hardangervidda (2004-2006: winter: 17, summer: 29, autumn: 19; including repeated approaches) (Table 1). Sight, alert and flight initiation distances increase with increasing encounter distance (Tables 2-4), indicating that when we approached reindeer from farther away they responded at longer distances (encounter distances 200 m and 400 m predicts sight distances at 166 m and 274 m and alert distances at 142 m and 228 m).

Controlling for other factors, sight distances were farther in winter than in summer and autumn, and within season farther in Hardangervidda compared to Blefjell (winter: 364 m *vs.* 263 m; summer: 292 m *vs.* 211 m; autumn: 246 m *vs.* 178 m; Fig. 2, Table 2). Sight distance was

Table 1. Untransformed response distances ± SE of wild reindeer disturbed by an intruding person in Blefjell and Hardangervidda (Hvidda) wild reindeer areas. All responses are distances measured in meters. The exception is assessment which is a binomial factor with "Yes" if the group was assessing the observer before flight (more than 1 second) and "No" for immediate flight.

Measurement	Encounter (Start)		Sight		Alert		Flight initiation		Escape (Distance moved)		Assessment	
	Hvidda	Blefjell	Hvidda	Blefjell	Hvidda	Blefjell	Hvidda	Blefjell	Hvidda	Blefjell	Hvidda	Blefjell
Mean	450	411	310	239	281	206	264	191	1545	482	Yes 19	12
SE	38	46	20	29	18	26	18	26	123	61	No 27	8
n	52	20	51	20	52	20	52	20	65	20	46	20

Table 2. Summary of the linear mixed-effects model for predicting sight distances (ln transformed) of groups of wild reindeer disturbed by an intruding person in Hardangervidda and Blefjell, south central Norway during three periods in 2004-2006. Reference levels for categorical variables are provided in the table (the level after the *vs.* term, such as "Blefjell" for area, "summer" for season and "level" for terrain).Values for ln encounter distance were centered around the mean.

Variable	Estimate	SE	df	*t*	*P*
Intercept	5.093	0.139	56	36.646	<0.001
Area (Hardangervidda *vs.* Blefjell)	0.324	0.092	56	3.503	0.001
Season (autumn *vs.* summer)	-0.171	0.097	56	-1.768	0.083
Season (winter *vs.* summer)	0.222	0.108	56	2.062	0.044
Encounter (Start) distance (ln m)	0.721	0.083	9	8.733	<0.001
Terrain (rugged *vs.* level)	0.260	0.105	9	2.484	0.035

Table 3. Summary of the linear mixed-effects model for predicting alert distances (ln transformed) of reindeer groups disturbed by an intruding person in Hardangervidda and Blefjell, south central Norway during three periods in 2004-2006. Reference levels for categorical variables are provided in the table (the level after the *vs.* term, such as "Blefjell" for area, "summer" for season and "level" for terrain).Values for ln encounter distance were centered at the mean.

Variable	Estimate	SE	df	*t*	*P*
Intercept	4.971	0.151	57	32.963	<0.001
Area (Hardangervidda *vs.* Blefjell)	0.386	0.100	57	3.845	<0.001
Season (autumn *vs.* summer)	-0.243	0.106	57	-2.302	0.025
Season (winter *vs.* summer)	0.152	0.116	57	1.313	0.195
Encounter (Start) distance (ln m)	0.683	0.090	9	7.576	<0.001
Terrain (rugged *vs.* level)	0.270	0.114	9	2.374	0.042

Table 4. Summary of the linear mixed-effects model for predicting flight initiation distances (ln transformed) of reindeer groups disturbed by an intruding person in Hardangervidda and Blefjell, south central Norway during three periods in 2004-2006. Reference levels for categorical variables are provided in the table (the level after the *vs.* term, such as "Blefjell" for area, "summer" for season and "level" for terrain).Values for ln encounter distance were centered at the mean.

Variable	Estimate	SE	df	*t*	*P*
Intercept	4.774	0.175	57	27.308	<0.001
Area (Hardangervidda *vs.* Blefjell)	0.416	0.116	57	3.575	0.001
Season (autumn *vs.* summer)	-0.170	0.123	57	-1.385	0.172
Season (winter *vs.* summer)	0.161	0.134	57	1.194	0.237
Encounter (Start) distance (ln m)	0.679	0.105	9	6.491	<0.001
Terrain (rugged *vs.* level)	0.348	0.132	9	2.638	0.027

Table 5. Summary of the linear mixed-effects model for predicting escape distances (ln transformed) of reindeer groups disturbed by an intruding person in Hardangervidda and Blefjell, south central Norway during three periods in 2004-2006. Reference levels for categorical variables are provided in the table (the level after the *vs.* term, such as "Blefjell" for area, "summer" for season and "females" for group structure). Values for ln encounter distance were centered at the mean.

Variable	Estimate	SE	df	*t*	*P*
Intercept	6.268	0.190	68	32.926	<0.001
Area (Hardangervidda *vs.* Blefjell)	1.125	0.153	68	7.349	<0.001
Season (autumn *vs.* summer)	-0.857	0.172	68	-4.977	<0.001
Season (winter *vs.* summer)	-0.582	0.189	68	-3.078	0.003
Group structure (males *vs.* females)	0.028	0.263	68	0.108	0.915
Group structure (mixed *vs.* females)	0.402	0.226	68	1.782	0.079

Table 6. Summary of the generalized linear mixed-effects model for predicting the probability of reindeer groups assessing an observer before fleeing. The response variable is binomially distributed (0=no assessment, 1= minimum one second assessment time). Reference levels for categorical variables are provided in the table (the level after the *vs.* term, such as "Blefjell" for area and "summer" for season.

Variable	Estimate	SE	*t*	*P*
(Intercept)	-0.928	0.668	-1.389	0.165
Area (Hardangervidda *vs.* Blefjell)	0.938	0.604	1.553	0.121
Season (autumn *vs.* summer)	1.499	0.690	2.172	0.030
Season (winter *vs.* summer)	-0.382	0.658	-0.581	0.561

Table 7. Genetic variation in 14 microsatellites in reindeer from Blefjell together with reference values of wild and semi-domestic reindeer herds from southern Norway. Values are number of individuals analysed (n), mean number of alleles per locus (A), mean allele richness (Ar) per locus, and mean genetic diversity (H).

Reindeer herd	n	A	Ar	H
1 Blefjell	24	5.1	4.9	0.68
Wild herds				
2 Nordfjella	24	7.2	6.8	0.76
3 Setesdal-Ryfylke	26	6.7	6.4	0.75
4 Hardangervidda	33	7.3	6.6	0.75
Semi-domestic herds				
5 Filefjell	30	6.4	5.9	0.72
6 Vågå	40	6.4	5.5	0.71
7 Røros	36	6.6	5.8	0.72

Table 8. Genetic differentiation among reindeer herds across 14 microsatellite loci. Pair wise values of F_{ST} are given above the diagonal and significance of differentiation in allele frequencies below the diagonal. Significant allele frequency differentiation (Benjamini-Hochberg corrected for multiple tests) is marked with asterisk.

Herd	1	2	3	4	5	6	7
1. Blefjell	-	0.053	0.081	0.078	0.083	0.088	0.094
2. Nordfjella	*	*	0.002	0.002	0.020	0.017	0.024
3. Setesdal-Ryfylke	*	*	-	0.004	0.029	0.030	0.034
4. Hardangervidda	*	ns	*	-	0.038	0.034	0.039
5. Filefjell	*	*	*	*	-	0.005	0.000
6. Vågå	*	*	*	*	*	-	0.009
7. Røros	*	*	*	*	ns	*	-

farther in rugged (364 m) than in level terrain (264 m; Table 2).

Groups became alert at longer distances in winter than in summers and autumn, and within season farther in Hardangervidda compared to Blefjell (winter: (323 m *vs.* 219 m; summer 278 m *vs.* 189 m; autumn 218 m *vs.* 148 m; Fig. 2, Table 3). Alert distances were longer in rugged (323 m) compared to level terrain (220 m).

Flight initiation distances were farther in winter than in autumn, and within season farther in Hardangervidda compared to Blefjell (winter: 298 m *vs.* 197 m; summer: 254 m *vs.* 167 m; autumn: 214 m *vs.* 141 m; Fig. 2, Table 4). Flight initiation distance was farther in rugged (299 m) than in level terrain (197 m; Table 4).

Escape distances were farther in summer than in winter and autumn, and within season farther in Hardangervidda compared to Blefjell (summer: 2428 m *vs.* 788 m; winter: 1357 m *vs.* 440 m; autumn: 1030 m *vs.* 334 m; Fig. 2, Table 5). There was a tendency for mixed groups to escape longer than females (Table 5).

The probability of assessing the observer before fleeing tended to be higher in Blefjell than in Hardangervidda (Fig. 3). Small sample size prevented a model including other factors than season (Table 6). Probability of assessing was lower in autumn than in winter and summer, and within season lower in Hardangervidda than in Blefjell (autumn18% *vs.* 36%; winter 59% *vs.* 79%; summer 49% *vs.* 79%).

Genetic analyses

All microsatellite loci, except for NVHRT-73 and RT-1 in the Blefjell herd, were in HWE after correcting for multiple tests. Both deviations were due to excess of homozygotes. The level of genetic variation was explicitly lower in the Blefjell reindeer as compared to the reference wild and semi-domestic herds with regard to the number of alleles observed, allele richness and gene diversity (Table 7) suggesting that some genetic loss has occurred during and after the founding of the Blefjell herds.

There was substantial genetic differentiation among the analysed herds as expressed by the F_{ST} (Table 8). The Blefjell herd were significantly different from all the reference herds

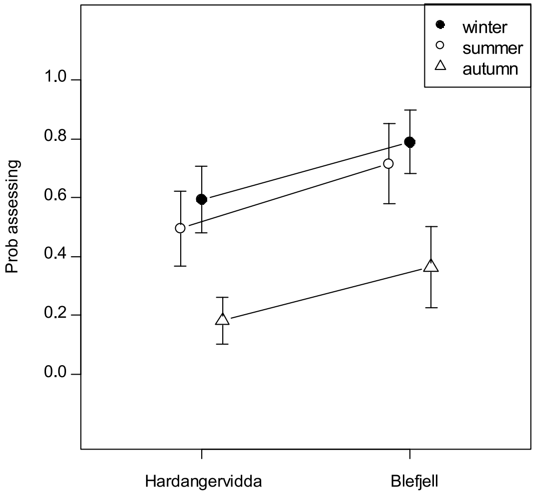

Fig. 3. The probability of assessing the observer before flight in reindeer groups in Blefjell and Hardangervidda south central Norway in three seasons. No categorical variables except season and no numerical variables were included in the model (see Table 6). Error bars are 1 SE.

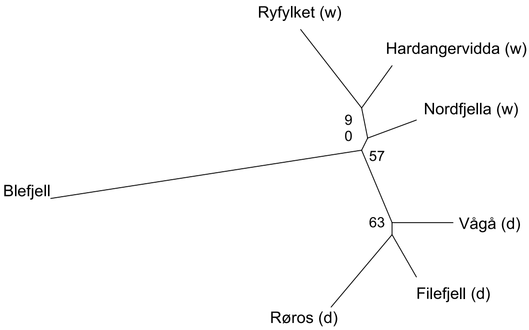

Fig. 4. Unrooted Neighbour Joining tree based on pairwise genetic distances ($D_{A)}$) between the Blefjell reindeer herd and three wild reindeer herds (w) and three semi-domestic reindeer herds (d). Bootstrap value of main branching is 100.

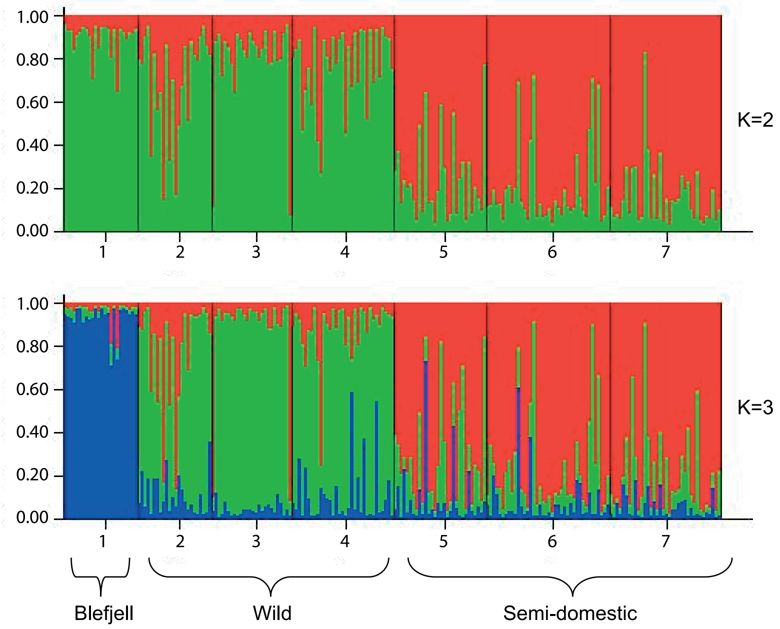

Fig. 5. Bayesian assignment of reindeer from Blefjell together with reference reindeer from three semi-domestic herds and three wild herds analyzed by SRUCTURE. Individual assignments are given to each of two (K = 2) and three (K = 3) clusters (different colours).

(mean F_{ST} ± SD of 0.080 ± 0.014) and showed more differentiated to the semi-domestic herds (mean F_{ST} = 0.088 ± 0.006) as compared to the wild herds (mean F_{ST} = 0.071 ± 0.016). For all comparison between the reference wild herds and the semi-domestic herds there were significant differences (mean F_{ST} = 0.030 ± 0.007), while generally low and partly non-significant differences were detected within both the semi-domestic herds (mean F_{ST} = 0.005 ± 0.005) and the wild herds (mean F_{ST} = 0.003 ± 0.001).

The un-rooted genetic distance (D_A) tree corroborated these results (Fig. 4). The semi-domestic herds clustered together different from the wild herds. Although, the long genetic distance of the Blefjell herd illustrates its genetic distinction, the branching gives a significant closer genetic distance of this herd towards the wild herds as compared to the semi-domestic herds, supporting a “wild” origin of the Blefjell reindeer herd.

The STRUCTURE algorithm showed a significant increase in log likelihood values (means ± SD across 10 repeats) from K = 1 (-9362.6 ± 9.3) to K = 2 (-9210.0 ± 9.9) and K = 3 (-9060 ± 7.7) after which the values dropped and were more unstable, suggesting presence of three populations. The proportionate memberships of individuals to these three clusters gave, besides those two dominated by respectively wild reindeer and semi-domestic (coloured green and red respectively in Fig. 5), also a separate cluster (coloured blue) consisted exclusively of Blefjell reindeer. When partitioning the material into two clusters, all reindeer from Blefjell showed main proportionate membership to the cluster dominated by the wild reindeer (Fig. 5) illustrating a closer ancestry of Blefjell reindeer towards the wild reindeer also at the individual level.

Discussion

While the encounter distance was the same in the two areas, the response distances sight, alert, flight initiation and escape were longer in Hardangervidda than in Blefjell, strongly indicating a decreased sensitivity to persons on foot or skies in the latter. Habituation relating to frequent encounters with humans and human infrastructure in the densely recreational Blefjell may explain the more relaxed behaviour in the Blefjell herd. The possible confounding effect from the introduced group of semi-domestic reindeer in 1961 is a critical alternative to the habituation hypothesis. The semi-domestic reindeer herds from different part of Scandinavia appear to be genetically very similar (eg Roed *et al.*, 2008). The possibility that the Blefjeld herd has an origin from some semi-domestic herd which is genetically quite different from the reference herds used here is therefore not likely. The reduced amount of genetic variation in the Blefjell herd together with its genetic distinctiveness when compared with the reference herds, suggests that genetic drift since founding of this herd some 40 years ago has been substantial. However, despite the genetic distinctiveness of the herd, the closer genetic ancestry to the wild herds as compared to the semi-domestic herds both at the herd and the individual level, supports the founding of this herd by wild reindeer from the Hardangervidda region and not from semi-domestic reindeer introduced to the region. This suggests that the differences in behaviour traits in Blefjell and Hardangervidda reindeer are not due to different influences of semi-domestic gene pools. Consequently, assuming that the founding individuals of the Blefjell was individuals with behaviour pattern typical for the origin herd, this study gives evidence that the different behaviour must have evolved during the period about thirty years since colonization of the Blefjell herd.

In both areas, seasonal hunting occurs during August and September. Most, but not all studies show that hunting results in behavioural changes that imply increase in alertness, flight initiation or escape distances (King & Workman, 1986; Bender *et al.*, 1999; de Boer *et al.*, 2004; Matson *et al.*, 2005; Donadio & Buskirk, 2006). Behrend & Lubeck (1968) concluded that properly controlled periodic autumn hunting of white-tailed deer (*Odocoileus virginianus*) in some New York parks may be compatible with summer viewing. In accordance with this, Colman *et al.* (2001) concluded that present-day hunting practices did not strongly affect the summer flight initiation distance of Svalbard reindeer (*R. t. platyrhynchus*) or habituation towards humans on foot. This conclusion is further supported by wild reindeer fright and flight response studies in southern Norway (Reimers *et al.*, 2009) and (E. Reimers *et al.*, in prep.). Kufeld *et al.* (1988) concluded that hunting pressure did not cause a change in dispersal of female mule deer (*O. hemionus*) or cause them to leave their normal home ranges.

An important influential factor is the frequency of interactions with humans (Louis & Le Beere, 2000; Tarlow & Blumstein, 2007). Ungulates in areas with frequent contact with humans showed reduced flight responses compared to those in areas where human contacts are rare (Denniston, 1956; Rowe-Rowe, 1974; Cassirer *et al.*, 1992; Colman *et al.*, 2001), which is in agreement with other taxa (Stankowich & Blumstein, 2005). In a recent paper Reimers *et al.* (2009) report that after 15 years of hunting the flight initiation distance in reindeer (pre-hunt *vs.* hunt) increased (40 ± 4 m to 81 ± 5 m) and fewer groups assessed the observer before taking flight (96% to 54%). Neither alert distance, escape distance, reindeer calf carcass weights nor the reindeer herd size changed during the length of the study (15 years). The frequent encounters between reindeer and humans in this highly tourist developed area prevented a predicted dramatic behavioural change following hunting. This behavioural effect most likely also applies to the Blefjell reindeer. Recreational use of Blefjell is, contrary to Hardangervidda, extensive due to densely populated surroundings, both in terms of permanent settlement, cabins, tourist resorts and year round hiking and skiing activities.

The behaviour of ungulates towards humans is likely to be the sum of the effects of all human activities (Jeppesen, 1987). While hunting has clear effects on flight responses in most species, non-consumptive recreation may buffer these effects and seasonal hunting may not impose enough negative stimuli towards humans to override habituation (Colman *et al.*, 2001; Reimers *et al.*, 2009). Several studies have shown that ungulates may not express behavioural differences in response to hunting if they also experience human in a non-threatening context (Behrend & Lubeck, 1968; Grau & Grau, 1980; Kufeld *et al.*, 1988) see also review by Stankowich (2008). Therefore, ungulates that routinely encounter humans in non-threatening context to which they can habituate, may only suffer minimal impacts on their behaviour towards humans if exposed to seasonal hunting.

Encounter distances were similar in the two areas. Sight, alert and flight initiation distances increased with the encounter distance indicating that when reindeer were encountered at long distances they responded at longer distances. Sight, alert and flight initiation distances tended to be longer in winter than in summer and shorter in autumn than in summer. This is in agreement with Reimers *et al.* (2006; 2009), who found that the observer is easier to detect when contrasted against snow and that rutting activities in autumn apparently lower the attention guard. Longer sight, alert and flight initiation distances in rugged than in level terrain most likely relate to the animals sense of control. Rugged terrain offers a lot more surprise options and hence a less predictive environment than the level terrain qualities. Reindeer exposed to stalking (hunting) movements after the hunting season did not exhibit increased levels of flight in response to a direct approach by a single human who paused only briefly. Rutting activities obviously affected reindeer behaviour more than the directly approaching observer during several October field-trial events which is in accordance with what we report from another wild reindeer area (Reimers *et al.*, 2006).

Conclusion

We conclude that habituation relating to frequent encounters with humans and human infrastructure in the densely human populated Blefjell explains the more relaxed behaviour of wild reindeer in the Blefjell herd compared to the reindeer in Hardangervidda, from which they originate.

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