

An experimental study of social attraction and spacing between the sexes in sheep

Pablo Michelena^{1,*}, Karine Henric¹, Jean-Marc Angibault², Jacques Gautrais¹, Paul Lapeyronie³, Richard H. Porter⁴, Jean-Louis Deneubourg⁵ and Richard Bon¹

¹Centre de Recherches sur la Cognition Animale, Université Paul Sabatier, F-31062, France, ²Laboratoire de Comportement et d'Ecologie de la Faune Sauvage, INRA, 31326 Castanet-Tolosan cedex, France, ³UMR Elevage des Ruminants en Région Chaude, Agro-M-INRA-CIRAD, 34060 Montpellier Cedex 1, France, ⁴Laboratoire de Comportement, INRA-CNRS, 37380 Nouzilly, France and ⁵Service d'Ecologie Sociale, Université Libre de Bruxelles, Bruxelles

*Author for correspondence (e-mail: michelen@cict.fr)

Accepted 29 September 2005

Summary

Most ungulates are gregarious species and outside the mating season are typically observed in single-sex groups. However little is known about the mechanisms underlying social segregation between sexes. We investigated the effect of conspecific attraction on individual spacing between unrestrained merino sheep *Ovis aries* and confined conspecifics. We considered differences between males and females and whether attractiveness of the confined conspecifics depends on their sex. A series of binary choice experiments was conducted in a large outdoor arena, located in pastures. One or two stimulus animals were placed in small individual cages (1.5 m×1 m) on opposite sides of the arena. Sheep were tested with one fixed peer of the same or opposite sex vs an empty cage, and with two fixed peers of either the same sex as themselves, or one male and one female. Sheep in a control condition were exposed to two empty cages. In all

of the test conditions, confined sheep were highly attractive. Males were more attracted by single stimulus peers of the same than the opposite sex, whereas females did not display such a preference. Sheep confronted with two restrained conspecifics tended to remain between the stimuli. This also occurred when the stimuli were of opposite sex, although the males tended to be located nearer the same-sex peer. Our findings can explain the strong aggregative behaviour of merino sheep, but also the social segregation previously observed in a mixed-sex group through higher attraction for same-sex than opposite-sex peers in males.

Key words: sheep, *Ovis aries*, attraction, spacing, sex discrimination, open field test.

Introduction

Most ungulates are gregarious species and are typically observed in groups that are flexible in size and composition. Individuals can join or leave conspecifics, resulting in fission (splitting) or fusion (amalgamation) of groups (Wilson, 1975; Gerard and Loisel, 1995; Conradt and Roper, 2000; Krause et al., 2000). The tendency to join or to leave groups depends on the attraction between individuals (Hamilton, 1971). In order to maintain group consistency, animals must synchronise their activity and motion and they must remain within an appropriate distance of neighbours (Deneubourg and Goss, 1989). However the mechanisms implicated in group dynamics of vertebrates, and in particular spacing of individuals, are poorly understood (Couzin et al., 2002; Krause and Ruxton, 2002).

In vertebrates, individuals of different age and sex classes do not mix randomly, but are assorted by phenotypes (Krause et al., 2000; Ward and Krause, 2001; Croft et al., 2003). In ungulates, individual animals tend to associate and/or interact primarily with conspecifics belonging to the same (social)

category, e.g. sex and age (Bon and Campan, 1996; see also Conradt, 1998). Social segregation between the sexes is a characteristic of the socio-spatial organization at a population level in social and dimorphic ungulates, and may result from several mechanisms, before and/or after fusion (Bon et al., 2005). For example, group fusion could be dependent upon the capacity of individuals to discriminate the activity or the phenotype of conspecifics at a distance. Weckerly et al. (2001) observed that Roosevelt elk *Cervus elaphus roosevelti* females avoid male groups exceeding six individuals. Bon and Campan (1996) proposed that social segregation between adults could result from social ontogeny – juvenile males being more motivated to interact than females using pseudo-sexual and agonistic-like interactions. As a result females may avoid or be indifferent to males. This would result in a social auto-segregation, i.e. males and females would prefer to associate with same-sex peers, which can have long-lasting effects during adulthood (Bon et al., 2001). Avoidance between sexes or a

preference for same-sex individuals would lead to a deficit of mixed-sex groups. Mathematical and theoretical models have been developed to demonstrate how internal group structure may result from an interplay between the social forces or motives, i.e. attraction and repulsion (Warburton and Lazarus, 1991; Guéron et al., 1996). However these models are not founded on experimental data and precise measures of the behaviour of animals are needed to validate them (Dumont and Boissy, 2000; Camazine et al., 2001; Couzin and Krause, 2003).

In freely moving groups, the interindividual distance can reflect different attraction or avoidance (Warburton and Lazarus, 1991; Whitehead, 1997). Moreover individual distances may vary seasonally, or with the sex, phenotype and other individual characteristics of the group members (Marler, 1956; Brown and Orians, 1970; Syme et al., 1975; Walther, 1977; Hinch et al., 1990; Stolba et al., 1990).

Most studies on individual distance have been performed on groups without taking into account the dynamics of the interactions between individuals (Keeling, 1995; Shiyomi and Tsuiki, 1999; Shiyomi, 2004). However, at any moment, inter-individual distances result from different possible interactions between the individual and there is no doubt that these interactions include stochastic components. Hence continuous recording of individual movements over a sufficient period of time is a prerequisite to understanding how individuals maintain a social distance to group members.

Pérez-Barbería et al. (2005) performed binary choice indoor experiments to study the social preferences of Soay sheep. They detected that adults preferred the individuals of their own sex. In the present study we attempted to gain further insights into these mechanisms involved in social segregation by testing social choice in relatively freeranging outdoor conditions, i.e. sheep were observed in the pasture where they graze. For this purpose, we registered the movement of freely moving individual merino sheep *Ovis aries* in an open field arena. In order to rule out the effect of movement and social interactions by conspecifics (e.g. affiliative, agonistic), we restrained their movement in space. We also measured the distances because (i) proximity can reflect attraction and social discrimination and (ii) the sexes may differ in 'social distance', and finally to assess how unrestrained sheep regulated the distance to conspecifics over time. Three series of binary choice experiments were performed with sheep of both sexes. In all tests the attractiveness of conspecifics was assessed by recording the pattern of movement of the test sheep. If sheep can discriminate the sex of conspecifics at a distance, we expected that they would move more readily towards confined individuals of the same sex rather than towards opposite sex animals. If a choice occurs at closer distances, the time spent near the same-sex should be longer than near the opposite-sex peer.

Materials and methods

Study area and subjects

The study was conducted at the experimental farm of

Domaine du Merle (5.74°E, 48.50°N) in the South of France. Subject and stimulus sheep *Ovis aries* Linnaeus were selected randomly from a group of merino males and a separate flock of females. Both male and female flocks were maintained in outdoor pastures and mixed only during the two annual mating periods (April–June and September–October). The experimental females and males were familiarised with each other by being housed together in a sheep barn for 2–5 weeks before the tests and were painted with a number on both flanks and on the rump. They were fed with Crau hay once a day and provided with water *ad libitum*.

Males bear horns and were heavier than ewes (mass 61.4±9.3 vs 49.4±5.4 kg, respectively, means ± S.D., Student's *t*-test $t_{64,62}=8.85$, $P<0.01$). At the time of testing, the mean age of the males and females was 3.7 (range=2–8) and 8.9 years (range=5–11), respectively.

Each ewe was treated with a vaginal sponge containing 30–40 mg of progesterone (replaced every 14 days) to block estrus and prevent any sexual interactions related to estrus. Animal care and experimental manipulations were in accordance with the rules of the French committee of animal experimentation ethics.

Experimental set-up and procedure

Individual sheep were tested with one confined stimulus peer of either the same or opposite sex vs an empty cage. In further experiments, test individuals were given a conflicting choice, i.e. confronted with two stimuli, one male/one female. Social attraction and sex discrimination were controlled by exposing sheep, respectively, to two empty cages and two confined peers of their own sex (Table 1).

Tests were conducted in 25 m arenas containing three wire cages (1.5 m×1 m×1 m). Two cages symmetrically located at the periphery of the arena were used as stimulus cages, containing a sheep or not, depending upon the treatment. The test sheep was placed into the third cage (releasing cage), located at the periphery of the arena, equidistant from stimulus cages. This cage was positioned on the side of the observation tower located at 22.5 m from the arena centre (Fig. 1). All test animals were familiar with the pastures as they regularly grazed on them throughout the year.

Because the test sheep grazed and depleted grass during the

Table 1. Effect of experimental conditions on the level of attraction and capability of discrimination in female and male merino sheep

Test sheep	Empty cages ¹	One stimulus sheep ²		Two stimulus sheep ³	
		SS vs EC	OS vs EC	SS vs OS	SS vs SS
Females	21	10	14	14	15
Males	9	16	14	14	14

EC, empty cage; SS, same sex; OS, opposite sex.

Values indicate the sample size for each condition.

¹Winter, 2004; ²summer, 2003; ³winter, 2003.

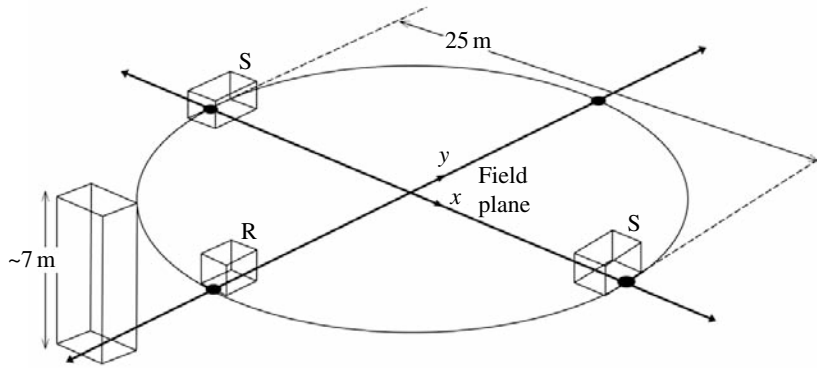


Fig. 1. Experimental design used to measure the level of attraction exerted by one or two stimulus conspecifics. R, releasing cage; S, stimulus cage. During the 30 min experiments, snapshots were taken at 1 min intervals using a digital camera anchored on the tower.

experiments, the arena was moved twice in winter 2003. Before each experiment, the test and confined animals, as well as their order of testing, were randomly selected. During each of the three periods of data collection (see below), all sheep were tested only once. Sheep were transported from the sheepfold to the arena in a trailer. During transport, which lasted 1–3 min, animals were blindfolded to reduce stress. In the tests requiring confined stimulus sheep, the latter were introduced first into a randomly selected cage. The test animal was then introduced into the releasing cage. One of the two observers remotely opened the door 3 min later from the top of the tower. Tests lasted 30 min, during which digital snapshots ($N=30$) were taken automatically at 1 min intervals, beginning 30 s after the test sheep was released, using a program of the Dimage 7i Minolta camera (Osaka, Japan).

Data collection

The experiments were carried out in winter and summer 2003 and in winter 2004 (see Table 1 for the chronology of experimental conditions). In winter, data were collected from 8:00 h to 18:00 h and in summer, from 6:00 h to 10:00 h and 18:00 h to 21:00 h because of animal inactivity (idling) during the highest temperatures of the day.

On each digital snapshot, the location of the test individual was visually tracked on a monitor using mouse click tracker software. Two points were specified corresponding to the on-ground projections of the hemi-distance between the forelegs and the hind legs. Then, classical 3D-reconstruction of object attitudes out of 2D-images was performed based on true perspective projection (Horn, 1999). The accuracy of sheep location was assessed by comparing the coordinates of sheep extracted from digital snapshots (c) to those obtained in the field by using a laser rangefinder (l) (Impulse model 200LR, Laser Technology Society Inc., Englewood, CO, USA). The coordinates obtained by each method were highly correlated, the estimated error on locations extracted from snapshot being <0.5 m (respectively x and y coordinated: $x_c=1.005 \times x_l - 0.123$, $r=0.99$, $N=226$, $P<0.001$ and $y_c=0.987 \times y_l - 0.196$, $r=0.99$, $N=226$, $P<0.001$).

Spatial analyses

We extracted several variables from the spatial distribution of each sheep, i.e. the area used, based on the 90% Convex

Polygon method using Ranges V software (Sargeant et al., 1993), the corresponding centroid, and the distance walked. This latter was approximated by the cumulated linear distances between consecutive locations.

Circularity of distribution was analysed using the Moore test (Zar, 1999). Lateral symmetry of the distribution in the arena without stimulus sheep was tested using a paired-data Student t -test. Attraction by conspecifics, supposed to result in a spatial skew, was tested by using a second order test of the significance of the mean (Zar, 1999).

We also considered the distribution of location density, computed by segmenting the arena into virtual 2 m rings (0–2 m, 2–4 m, etc) centred on the centre of the side of both cages adjacent to the arena. In order to compare the social conditions, the distributions of location density were normalised by the total number of locations.

We predicted that if the stimulus sheep exerted a strong social attraction, the distribution of location density of test sheep should be biased towards the corresponding cage, and the part of arena explored (i.e. area used and distances walked) would be small compared to random movement in the arena without social attraction.

The minimum distances to the cages within the first 4 min after release were also compared. This interval of time was chosen because the distance of approach to stimulus cages was smaller than the distance of the centroid of locations over the 30 min period for 94% of test animals.

Activity budget of test sheep

In freely moving groups, sheep mainly engage in feeding when active, therefore the distance between individuals reflects movements during grazing. We were interested to study social attraction between individuals in pasture conditions. However the stress associated with isolation could modify the behaviour of sheep. We therefore recorded the activity of test animals continuously using a Thomson dk 52 micro cassette recorder (Stanford, CT, USA) to measure their time spent grazing, standing, walking, lying/idling and bleating. From all animal tested ($N=176$), 24 were discarded: 2 ewes escaped from the arena, 3 males entangled their horns in the fence net, 2 animals were lying down throughout the test, 1 stayed in the releasing cage and 16 were idle (this occurred only in summer). Since the remaining sheep did not leave the releasing cage

Table 2. General activity of test sheep measured in the set-up to study social attraction and spacing

Test trial	Males					Females				
	Grazing	Standing, head up	Moving	Bleating	N	Grazing	Standing, head up	Moving	Bleating	N
SS vs EC	95 (94–97) ^a	2 (1–5) ^a	1 (0–1) ^a	0.14 ^b	15	76 (57–83) ^a	17 (11–27) ^a	3 (2–9) ^a	0.89 ^b	9
OS vs EC	90 (83–94) ^a	8 (1–15) ^a	0 (0–1) ^a	0.07 ^b	14	73 (64–86) ^a	20 (12–29) ^a	3 (1–8) ^a	0.93 ^b	14
SS vs SS	96 (93–97) ^a	2 (1–6) ^a	1 (0–1) ^a	0.05 ^b	19	88 (79–90) ^a	10 (7–16) ^a	1 (0–3) ^a	0.35 ^b	17
SS vs OS	92 (83–96) ^a	4 (1–9) ^a	2 (0–4) ^a	0.07 ^b	14	80 (75–92) ^a	15 (6–21) ^a	2 (0–6) ^a	0.14 ^b	14
EC vs EC	82 (67–93) ^a	15 (6–22) ^a	1 (0–4) ^a	0.08 ^b	10	51 (30–61) ^a	36 (23–44) ^a	13 (10–20) ^a	0.86 ^b	21

EC, empty cage; SS, same sex; OS, opposite sex.
^aMedian proportion of time, percentiles 25–75% are given in parentheses.
^bPercent of test sheep bleating.
N, number of test sheep in each trial.

instantaneously, we analysed the proportion of time engaged in each activity. Because test sheep were mainly engaged in feeding activity, to detect possible outliers we only considered the time spent feeding. The proportion of time spent grazing (*P*) did not follow a normal distribution, and was thus transformed using the $\ln[\arcsin\sqrt{P}]$. The Dixon Extreme Value test (Dixon, 1953) was used to detect outliers of an assumed normal distribution of the grazing time. The Dixon test identified 5 outliers, which were discarded from the analyses. Statistical tests were performed with SPSS software. All reported values are means \pm s.d.

Results

Bleats by stimulus animals and location of test sheep

Among all test animals, 63% of females and 8% of males were heard to emit bleats. The frequency of bleats was highly variable (range: 1–85 bleats by experiments, median=20) and the sex of the stimulus sheep showed no effect on the proportions of bleating test sheep (Fischer exact test, all $P \geq 0.24$; Table 2). Among the stimulus animals, 72% of females and 28% of males emitted bleats.

In experiments with one stimulus sheep, when considering the 1 min interval centred on each location, test subjects remained more distant from the confined sheep when it was bleating than when it was silent (distance 11 ± 4.7 m vs 8.1 ± 4.1 m, means \pm s.d.; paired sample *t*-test: $t_{27}=4.7$, $P < 0.01$). Further analysis revealed that the probability of bleating by the stimulus animal increased linearly with the distance from the test subject: $y=0.0194x+0.0922$ ($r=0.95$, $N=13$, $P < 0.01$). To detect whether bleating by stimulus sheep attracted the test animals, we calculated the difference of distance between both sheep at time *t* and *t*+1 min ($N=29$). A negative difference indicates that test sheep approached the cage, while a positive one indicates that it moved away. We then calculated the ratio of the number of approaches to the total number of movements (approach + withdrawal), when the stimulus sheep bleated, and when it did not, during the corresponding 1 min interval for each test sheep. Comparison of the distribution of these two ratios revealed that the confined sheep's bleating did not

modify the probability of approach by the test sheep (ratio with bleats: 0.51 ± 0.23 , without bleats: 0.48 ± 0.22 , paired sample *t*-test: $t_{23}=0.45$, $P=0.66$).

General activity of test sheep

When the door of the releasing cage was opened, 70% of test sheep immediately left the cage and 91% exited the cage within the 30 first seconds (8 ± 3 s, range 0–98 s). The sex of the stimulus sheep had no significant effect on the proportion of instantaneous exits for either females or males (females: one stimulus sheep of the same and opposite sex, respectively: 0.13 vs 0.31, Fisher exact test: $N=23$, $P=0.39$; two stimulus sheep: 0.26 vs 0.36, $N=31$, $P=1$; males: one stimulus sheep: 0.25 vs 0.29, $N=29$; two stimulus sheep: 0.32 vs 0.46, $N=33$, both $P=1$). When pooling data, no significant differences between males and females were found in the proportion of instantaneous exits with no stimulus sheep (χ^2 test, 0.43 vs 0.23, $\chi^2_1=1.63$, $P=0.2$), with one stimulus (0.27 vs 0.23, $\chi^2_1=0.14$, $P=0.71$) or with two stimulus sheep (0.37 vs 0.3, $\chi^2_1=0.38$, $P=0.54$).

In the arena, all test sheep spent more than 75% of the 30 min grazing (median=88%; Table 2). When not grazing, the most common activity was standing with head up (9%); 25% of test animals were never observed walking and those that did walk spent little time on this activity (median=2%).

When alone, males spent more time grazing than females ($t_{29}=3.19$, $P < 0.01$; Table 2). Two-way ANOVAs revealed that the sex of stimulus sheep did not significantly affect the time spent grazing by test sheep (one stimulus sheep: $F_{1,52}=0.87$, $P=0.36$; two stimulus sheep: $F_{1,64}=1.72$, $P=0.2$). However, the sex of test sheep was significant: males spent more time grazing than females (one stimulus sheep: $F_{1,52}=36.7$, $P < 0.01$; two stimulus sheep: $F_{1,64}=21.6$, $P < 0.01$). No significant interactions were found (one fixed: $F_{1,52}=1.72$, $P=0.2$; two fixed: $F_{1,64}=1.3$, both $P=0.26$).

Spatial analyses

Experiments with no stimulus sheep

In the tests with two empty cages, both males and females walked relatively large distances and explored on average 33%

Table 3. Proportion of locations in each half-arena, distance of centroid of location and of approach (nearest location) to the cages, total distance walked and area explored by test sheep

Test sheep	Stimulus cages	Location		Distance				d.f.	Distance walked (m)	Area used (m ²)
		%	<i>t</i>	Centroid (m)	<i>t</i>	Nearest location (m)	<i>t</i>			
Males	SS	92±17	-9.5	5.6±3.3	-9.2	4.2±5.8	-6.4	14	55±23	35±35
	EC	8	(<0.001)	20.7±3.1	(<0.001)	18.3±4.1	(<0.001)			
	OS	80±28	-4.1	9.5±3.2	-4.7	6.9±4.9	-3.2	13	67±21	64±37
	EC	20	(0.001)	17.1±3.1	(<0.001)	15.9±4.9	(0.01)			
	SS left	43±27	1.1	13.9±3.2	1.2	12±6	0.5	18	78±31	115±57
	SS right	57	(0.28)	12.1±3.6	(0.24)	11.1±6.6	(0.6)			
	SS	61±29	-1.4	11.6±3.9	-1.3	11.6±4.8	-0.8	13	102±40	132±58
	OS	39	(0.17)	14.4±3.9	(0.2)	13.1±4.7	(0.46)			
	EC left	42±32	0.8	14.6±5	1	13.9±3	0.9	9	113±59	140±65
	EC right	58	(0.45)	11.6±4.8	(0.35)	12.1±4.6	(0.37)			
Females	SS	79±27	-3.3	7.8±4.5	-3.7	3.9±6.8	-3.4	8	144±98	110±91
	EC	21	(0.01)	18.4±4.2	(0.006)	17.8±6.5	(0.01)			
	OS	85±19	-6.8	7.6±3.9	-5.3	2.7±3.1	-12.4	13	123±86	102±103
	EC	15	(<0.001)	18.7±3.9	(<0.001)	19.3±2	(<0.001)			
	SS left	54±29	-0.6	12.5±4.2	-0.3	10.4±6.7	0.05	16	106±83	102±58
	SS right	46	(0.55)	13.2±4.5	(0.74)	10.2±5.9	(0.96)			
	SS	58±28	-1	12.7±3.2	-0.6	10.3±5.5	-1.3	13	98±41	111±63
	OS	42	(0.33)	13.8±3.2	(0.54)	12.9±5.2	(0.21)			
	EC left	38±16	3.5	14.2±2	2.5	8.8±5.1	0.4	20	196±70	186±65
	EC right	62	(0.002)	12±2.4	(0.02)	8±5.1	(0.69)			

EC, empty cage; SS, same sex; OS, opposite sex.

Values are mean ± s.d. of the following variables: % of locations in each half-arena (%); distance of the centroid over the 30 min period (centroid); minimum distance to the cages within the first 4 min (nearest location); total distance walked and area explored.

t indicates results of one sample and paired *t*-tests comparing, respectively, the proportion of locations and the distances to both cages.

Probabilities (*P*) are given in parentheses; d.f., degrees of freedom.

of the arena (Table 3). Females dispersed more rapidly in the arena, walked longer distances (Student *t*-test, $t_{29}=-3.24$, $P<0.01$) and tended to use larger areas ($t_{29}=-1.86$, $P=0.07$) than males. The distribution of female locations relative to the cages was similar to a random distribution (Fig. 2A). Males tended to be located more often at the periphery of the arena but they were uniformly distributed in each angular sector (Moore test of circular uniformity: $R'=0.89$, $N=10$, $P=0.20$). They were found as often in the left and right halves of the arena and at similar distances from the left and right cages (Table 3). Female distribution was slightly biased ($R'=1.23$, $N=21$, $P<0.05$) as they more often used the angular sector of the releasing cage (mean angular deviation to the releasing cage: $\Delta_m=35^\circ$, second order test of the significance of the mean: $F_{2,19}=3.47$, $P=0.05$). They also occurred more often in the right than in the left half arena ($62\pm 16\%$ of locations). However, no asymmetry in female location was detected when considering the centroid of locations (Table 3). Mean distance from the cages remained stable over the 30 min test trial, except during the first 5 min within which sheep of both sexes

slowly dispersed in the arena from the releasing cage (distance to the nearest cage, respectively, during 0–5 min, 5–15 min and 15–29 min: 10.3 ± 3.1 m, 9 ± 2.2 m and 9.2 ± 2.3 m).

Experiments with one stimulus sheep

The distributions of location density with one stimulus sheep differed markedly from that obtained with no stimulus conspecific (Fig. 2B,C). Test sheep of both sexes stayed close to the stimulus sheep regardless of its sex, being most often located in the corresponding half-arena (Table 3). The attraction to the stimulus sheep was clearcut during the experiment. During the first 4 min following the release, sheep while grazing approached the cage containing the stimulus more rapidly than in the experiments with empty cages (see nearest location in Table 3). Afterward, they diffused in the arena. Beyond these first minutes, sheep tended to remain at a constant distance to the stimulus cage (distance to the nearest cage during 0–5 min: 6.6 ± 4 m; 5–15 min, 6.8 ± 3.3 m and 15–30 min, 7.5 ± 3.2 m). This was also observed in experiments without conspecifics, but the steady state was achieved later

and sheep were farther from the cages than in experiments with one stimulus.

In the presence of one stimulus sheep, males' locations were concentrated in the corresponding angular sector, regardless of whether the stimulus was a male (Moore test for circular uniformity respectively, $R'=1.97$, $N=15$, $P<0.05$; mean angular deviation from the stimulus cage: $\Delta_m=11^\circ$, second order test of the significance of the mean: $F_{2,13}=44.95$, $P<0.05$) or a female ($R'=1.54$, $N=14$, $P<0.05$; $\Delta_m=-23^\circ$, $F_{2,12}=10.36$, $P<0.05$). Similar results were found for females (female stimulus: $R'=1.33$, $N=9$, $P<0.05$; $\Delta_m=20^\circ$; $F_{2,7}=6.64$, $P<0.05$; male stimulus: $R'=1.76$, $N=14$, $P<0.05$; $\Delta_m=-20^\circ$, $F_{2,12}=14.75$, $P<0.05$).

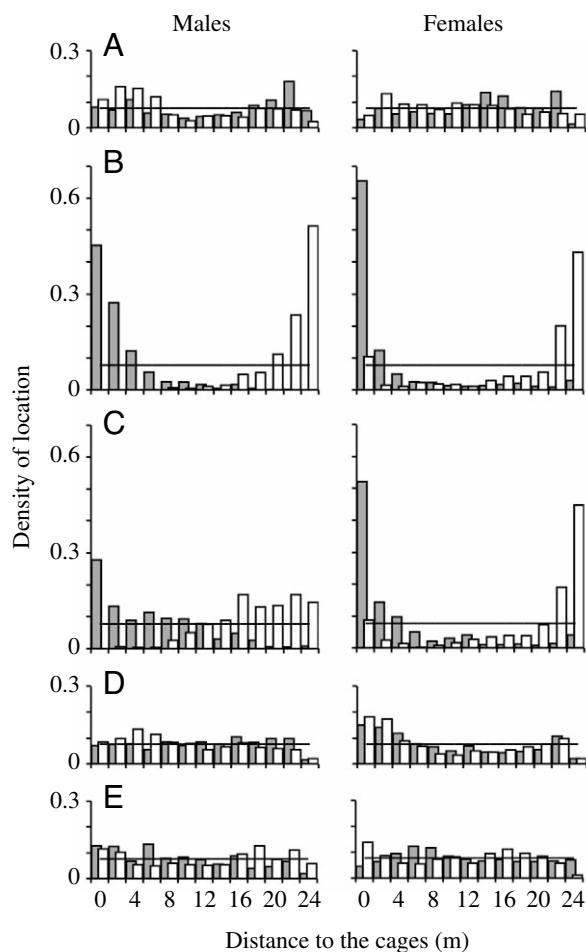


Fig. 2. Distribution of the observed (bars) and expected (horizontal line) probability of presence in 2 m rings of test males and females as a function of the distance to the cages. The observed probability is the proportion of the locations/2 m ring surface. The expected probability is calculated under the hypothesis of random distribution of sheep. Gray bars represent the distributions relative to the same-sex (B) and opposite-sex stimulus (C) and white bars to the empty cage. When confronted with two empty cages (A) or two same-sex stimuli (D) the grey and white bars represent, respectively, to the right and left cages. With two opposite-sex stimuli (E), grey bars represent the distribution relative to the stimulus of same sex as the test sheep, and white bars to the opposite sex.

Within the first min, test females approached the stimulus sheep, whatever its sex, at closer distances than males. The latter approached the stimulus males more closely than the females. No significant differences between sexes were found regarding the distance of the centroid of location to the stimulus cage. However the sex of stimulus sheep and the interaction sex of test sheep \times sex of stimulus were near significance. Males' locations (centroids) were closer to the stimulus cage when it contained a male than a female. In addition, males walked shorter distances and spread less than females. Test males also used smaller areas and walked less in the presence of a stimulus of the same sex than of the opposite sex (Tables 3, 4).

Experiments with two stimulus sheep

The distributions of density location with two stimulus and no stimulus sheep were similar (Fig. 2D,E). After the first minutes following release, when the sheep spread out, the mean distances to cages remained stable (distance to the nearest cage during 0–5 min, 10.3 ± 3.5 m; 5–15 min, 8.2 ± 2 m and 15–30 min, 8.2 ± 2 m), closer than in the tests with empty cages but larger than when only one stimulus sheep was present. Females and males used a smaller area and walked less with two stimulus sheep than without any. One additional major difference with the one-stimulus experiments was that test sheep did not approach any of the stimuli as closely either during the first 4 min or during the entire 30 min session (Table 3).

In contrast to what we expected, the presence of a same-sex sheep among the two stimuli did not markedly affect the distribution and behaviour of test animals, except that test females and males tended to graze nearer to the same-sex than to the opposite-sex stimulus over the 30 min (Table 3). Males also walked more and used a larger area when a female stimulus was present than when not. Males and females were found as often in both halves of the arenas when tested with two same-sex and opposite-sex stimuli. The angular distribution of females was uniform (Moore test for circular uniformity: $R'=0.83$, $N=17$, $P=0.17$) when confronted with two females, whereas they were more often located in the sector of the releasing cage when confronted with opposite-sex stimuli ($\Delta_m=16^\circ$; $F_{2,12}=7.45$, all $P<0.05$). Males also occurred more often in the sector corresponding to the releasing cage in both social contexts (same-sex: $R'=1.18$, $N=19$, $P=0.03$; mean angular deviation to the releasing cage: $\Delta_m=-26^\circ$; second order test of the significance of the mean: $F_{2,17}=6.15$; opposite-sex: $R'=1.36$, $N=14$, $P<0.01$; $\Delta_m=27^\circ$; $F_{2,12}=10$, $P<0.01$).

Discussion

The primary goal of this study was to elucidate the dynamics of social attraction between merino sheep in conditions close to those faced by free-ranging groups. During the experiments, the latter never lay down. We also aimed to elucidate the effects of gender on inter-individual distance in merino sheep. Sheep were observed in an outdoor arena that was large enough

Table 4. Two-way ANOVAs of spatial behaviour of test sheep as a function of their sex and the sex of stimulus animals

Test trial	Spatial variables	Sex effect		Interaction
		Test sheep	Confined sheep	
One stimulus (<i>N</i> =52)	Distance of the centroid	0.01 (0.94)	3.16 (0.08)	3.75 (0.06)
	Distance of the nearest location	5.74 (0.02)	5.3 (0.03)	1.37 (0.25)
	Distance walked	17.22 (<0.001)	0.06 (0.8)	0.87 (0.35)
	Area used	8.02 (0.01)	0.28 (0.6)	0.9 (0.35)
Two stimulus (<i>N</i> =64)	Distance of the centroid	0.1 (0.76)	0.51 (0.48)	0.88 (0.35)
	Distance of the nearest location	1.28 (0.26)	6.01 (0.02)	0.7 (0.4)
	Distance walked	0.6 (0.44)	1.36 (0.25)	1.72 (0.19)
	Area used	1.38 (0.24)	0.76 (0.39)	0.07 (0.79)

F-values of main factors of sex of test sheep, sex of stimulus (or combination of stimulus) and of their interaction are indicated. Probabilities (*P*) are given in parentheses.

to allow inter-individual distances that are commonly reported for flocks of this breed (Dudzinski and Schuh, 1978; Arnold, 1985). Experiments with one restrained peer revealed that conspecifics exerted a strong attraction. Within minutes of being released, the test sheep readily moved towards the stimulus peer, regardless of its sex. Thereafter, the analyses of symmetry and distance of locations to the cages show that sheep remained close to the stimulus animal over the 30 min experiment. They also used smaller areas than when tested with two empty cages. These results confirm previous studies indicating that merino sheep are highly gregarious (Arnold and Pahl, 1974; Arnold et al., 1981). In addition the observed inter-individual distances were close to those reported within merino flocks (Arnold, 1985; Michelena, 2001).

Sex differences were observed in the behaviour of the subject sheep. Males remained closer to peers of their own sex. They also spent less time grazing and more time standing head-up when a female was present in the arena, regardless of whether a confined male was also present. Sex-specific attraction by females was unclear since they tended to remain at equivalent distances from male and female peers. This does not necessarily imply that ewes cannot discriminate the sex of conspecifics (Kendrick et al., 1995; Gelez et al., 2004; Pérez-Barbería et al., 2005). Females seemed more stressed than males, reflected in longer distances walked and more frequent vocalisations. However, conspecifics exerted a clear attraction. Whether stress provoked by the experiments could explain the indiscriminate female behaviour is unclear: why did females not reduce the interindividual distance to reduce distress? These results therefore only partly support the social affinity hypothesis (Bon et al., 2005).

When confronted with two stimulus peers, sheep displayed clear differences in behaviour when compared to the tests with a single confined peer. They approached the stimulus sheep less closely and spent more time at an intermediate distance between the two cages in the former than in the latter situation. Their oscillating movements probably reflect conflicting motives to approach one peer while remaining not too far from the second one, so limiting their walking. This was perhaps possible because by doing so they were still within, or near,

the limit of the social distance. The behaviour of the males when confronted with one or two stimulus males was striking. Contrary to what was expected and found by Pérez-Barbería et al. (2005), the presence of a same-sex peer among the two fixed animals did not substantially modify the subjects' behaviour. The ability to move in a large pasture area perhaps explains why no clearcut choice was found, as in experiments with only one stimulus peer. Whether social choice depends on the distance between stimulus cages or on the possibility of moving around the cages remains to be tested.

The results we found in the presence of two peers suggest that within this distance scale, merino sheep stayed at mid-distance from both peers and thus limited group splitting. We previously observed a group containing 15 adults of both sexes for 7 weeks. While grazing, merinos congregated in a very small surface area, which may reflect strong inter-individual attraction of sheep, limiting spreading of the group (Michelena et al., 2004). With respect to that study, we also note that individual distances were close to the individual distance measured in the present experiment (see also Michelena, 2001). The stability of the mixed-sex groups might be explained by a similar level of attraction of both sexes for females. However, we also found that pairs of sheep of the same sex were more frequent than expected (Michelena et al., 2004). Social discrimination of males, as reflected by inter-individual distance, is one factor that may have played a role in this social segregation on a small scale. However, other behavioural or social mechanisms, such as differences in activity budget, movement speed, or avoidance of males by females, may contribute to social segregation. Further experiments are needed to explore how the number of freely moving individuals and possibly other mechanisms interact to influence spacing.

Various authors have hypothesized the existence of an individual personal zone within which repulsion is higher than attraction to peers (Moody et al., 1997; Shiyomi and Tsuiji, 1999; Couzin et al., 2002; Shiyomi, 2004). Our findings suggest, however, that the repulsion is very low or nil, taking into account that the sheep were not able to engage in agonistic interactions and that the stimulus sheep could not avoid the test subjects. In these experimental conditions, it is not possible to determine

whether movement away from the confined peer corresponds to repulsion or to search for food combined with the inertia of walking. The inter-individual distance does not appear to be the result of a balance between attraction and repulsion forces, but rather an effect of attraction exerted by peers on the exploratory behaviour and mobility rate of grazing sheep.

We are pleased to acknowledge the staff of Domaine du Merle for technical advice and assistance. We thank Denis Vauthier (INRA, Unité de Recherche Forestière Méditerranéenne) for the use of the telemeter, Bruno Cargnelutti for assistance with RANGES V, and Baptiste Bonnière-Michelena, Virginia Colom, Severine Ligout, Sarah Noël and Coralie Tacquet for help and assistance during the experiments. This research was supported by the European LEURRE project, a French-Belgium Tournesol Project and a Projet Fléché from the Institut National de la Recherche Agronomique. P.M. was supported by a Belgium Tournesol grant. J.G. and P.M. were supported by the LEURRE Project sponsored by the Future and Emerging Technologies program of the European Community (IST-2001-35506).

References

- Arnold, G. W. (1985). Territoriality. In *Ethology of Farm Animals* (ed. A. F. Fraser), pp. 249-264. Amsterdam: Elsevier.
- Arnold, G. W. and Pahl, P. J. (1974). Some aspects of social behaviour in domestic sheep. *Anim. Behav.* **22**, 592-600.
- Arnold, G. W., Wallace, S. R. and Rea, W. A. (1981). Associations between individuals and home-range behaviour in natural flocks of three breeds of domestic sheep. *Appl. Anim. Ethol.* **7**, 239-257.
- Bon, R. and Campan, R. (1996). Unexplained sexual segregation in polygamous ungulates: a defense of an ontogenetic approach. *Behav. Process.* **38**, 131-154.
- Bon, R., Rideau, C., Villaret, J.-C. and Joachim, J. (2001). Segregation is not only a matter of sex in Alpine ibex, *Capra ibex ibex*. *Anim. Behav.* **62**, 495-504.
- Bon, R., Deneubourg, J.-L., Gerard, J.-F. and Michelena, P. (2005). Sexual segregation in ungulates: from individual mechanisms to collective patterns. In *Sexual Segregation in Vertebrates* (ed. K. Ruckstuhl and P. Neuhaus), pp. 180-199. Cambridge: Cambridge University Press.
- Brown, J. L. and Orians, G. H. (1970). Spacing patterns in mobile animals. *Annu. Rev. Ecol. Syst.* **1**, 239-262.
- Camazine, S., Deneubourg, J.-L., Franks, N. R., Sneyd, J., Theraulaz, G. and Bonabeau, E. (2001). *Self-Organization in Biological Systems*. Princeton, New Jersey: Princeton University Press.
- Conradt, L. (1998). Could asynchrony in activity between the sexes cause intersexual social segregation in ruminants? *Proc. R. Soc. Lond. B* **265**, 1359-1363.
- Conradt, L. and Roper, T. J. (2000). Activity synchrony and social cohesion: a fission-fusion model. *Proc. R. Soc. Lond. B* **267**, 2213-2218.
- Couzin, I. D. and Krause, J. (2003). Self-organization and collective behavior in vertebrates. *Adv. Study Behav.* **32**, 1-75.
- Couzin, I. D., Krause, J., James, R., Ruxton, G. D. and Franks, N. R. (2002). Collective memory and spatial sorting in animal groups. *J. Theor. Biol.* **218**, 1-11.
- Croft, D. P., Arrowsmith, B. J., Bielby, J., Skinner, K., White, E., Couzin, I. D., Magurran, A. E., Ramnarine, I. and Krause, J. (2003). Mechanisms underlying shoal composition in the Trinidadian guppy (*Poecilia reticulata*). *Oikos* **100**, 429-438.
- Deneubourg, J.-L. and Goss, S. (1989). Collective patterns and decision making. *Ethol. Ecol. Evol.* **1**, 295-311.
- Dixon, W. J. (1953). Processing data for outliers. *Biometrics* **9**, 74-89.
- Dudzinski, M. L. and Schuh, H. J. (1978). Statistical and probabilistic estimators of forage conditions from grazing behaviour of merino sheep in a semi-arid environment. *Appl. Anim. Ethol.* **4**, 357-368.
- Dumont, B. and Boissy, A. (2000). Gazing behaviour of sheep in situation of conflict between feeding and social motivations. *Behav. Process.* **49**, 131-138.
- Gelez, H., Archer, E., Chesneau, D., Campan, R. and Fabre-Nys, C. (2004). Importance of learning in the response of ewes to male odor. *Chem. Senses* **29**, 555-563.
- Gerard, J.-F. and Loisel, P. (1995). Spontaneous emergence of a relationship between habitat openness and mean group size and its possible evolutionary consequences in larger herbivores. *J. Theor. Biol.* **176**, 511-522.
- Gueron, S., Levin, S. A. and Rubenstein, D. I. (1996). The dynamics of mammalian herds: from individuals to aggregations. *J. Theor. Biol.* **182**, 85-98.
- Hamilton, W. D. (1971). Geometry of the selfish herd. *J. Theor. Biol.* **31**, 295-311.
- Hinch, G. N., Lynch, J. J., Elwin, R. L. and Green, G. C. (1990). Long-term associations between Merino ewes and their offspring. *Appl. Anim. Behav. Sci.* **27**, 93-103.
- Horn, B. K. P. (1999). Projective geometry considered harmful: <http://www.ai.mit.edu/people/bkph/publications.html>.
- Keeling, L. (1995). Spacing behaviour and an ethological approach to assessing optimum space allocations for group of laying hens. *Appl. Anim. Behav. Sci.* **44**, 171-186.
- Kendrick, K. M., Atkins, K., Hinton, M. R., Road, K. D., Fabre-Nys, C. and Keverne, B. (1995). Facial and vocal discrimination in sheep. *Anim. Behav.* **49**, 1665-1676.
- Krause, J. and Ruxton, G. D. (2002). *Living in Groups*. Oxford: Oxford University Press.
- Krause, J., Hoare, D. J., Croft, D., Lawrence, J., Ward, A., Ruxton, G. D., Godin, J.-G. and James, R. (2000). Fish shoal composition: mechanisms and constraints. *Proc. R. Soc. Lond. B* 2011-2017.
- Marler, P. (1956). Studies of fighting in chaffinches proximity as a cause of aggression. *Brit. J. Anim. Behav.* **4**, 23-30.
- Michelena, P. (2001). Mécanismes sociaux et comportementaux générant la ségrégation des sexes chez les ongulés: le cas du mouton mérinos. DEA, Université Paul Sabatier, Toulouse, France.
- Michelena, P., Bouquet, P. M., Dissac, A., Fourcassié, V., Lauga, J., Gerard, J.-F. and Bon, R. (2004). An experimental test of hypotheses explaining social segregation in dimorphic ungulates. *Anim. Behav.* **68**, 1371-1380.
- Moody, A. L., Thompson, W. A., De Bruijn, B., Houstons, A. I. and Goss-Custard, J. D. (1997). The analysis of spacing of animals, with an example based on oystercatchers during the tidal cycle. *J. Anim. Ecol.* **66**, 615-628.
- Pérez-Barbería, F. J., Robertson, E. and Gordon, I. J. (2005). Are social factors sufficient to explain sexual segregation in ungulates? *Anim. Behav.* **69**, 827-834.
- Sargeant, A., Raymond, B., Greenwood, J., Sovada, M. A. and Shaffer, T. L. (1993). Distribution and abundance of predators that affect duck production – Prairie Pothole Region. Resource Publication 194. Jamestown: US Fish and Wildlife Service.
- Shiyomi, M. (2004). How are distances between individuals of grazing cows explained by a statistical model? *Ecol. Model.* **172**, 87-94.
- Shiyomi, M. and Tsuki, M. (1999). Model for the spatial pattern formed by a small herd in grazing cattle. *Ecol. Model.* **119**, 231-238.
- Stolba, A., Hinch, G. H., Lynch, J. J., Adams, D. B., Munro, R. K. and Davies, H. I. (1990). Social organization of Merino sheep of different ages sex and family structure. *Appl. Anim. Behav. Sci.* **27**, 337-349.
- Syme, L. A., Syme, G. J., Waite, T. G. and Pearson, A. J. (1975). Spatial distribution and social status in a small herd of dairy cows. *Anim. Behav.* **23**, 609-614.
- Walther, F. R. (1977). Sex and activity dependency of distances between Thomson's Gazelles (*Gazella thomsoni gunther* 1884). *Anim. Behav.* **25**, 713-719.
- Warburton, K. and Lazarus, J. (1991). Tendency distance models of social cohesion in animals groups. *J. Theor. Biol.* **150**, 473-488.
- Ward, A. J. and Krause, J. (2001). Body length assortative shoaling in the European minnow. *Anim. Behav.* **62**, 617-621.
- Weckerly, F. W., Ricca, M. A. and Meyer, K. P. (2001). Sexual segregation in Roosevelt elk: Cropping rates and aggression in mixed-sex groups. *J. Mammal.* **82**, 825-835.
- Whitehead, H. (1997). Analysing animal social structure. *Anim. Behav.* **53**, 1053-1067.
- Wilson, E. O. (1975). *Sociobiology*. Harvard: Harvard University Press.
- Zar, J. H. (1999). *Biostatistical Analysis*. New Jersey: Prentice-Hall.