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CICLO XXIII

BEHAVIOURAL AND GENETIC INVESTIGATION OF FIGHTING ABILITY IN VALDOSTANA BREED

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La bataille di vatse a Vertozan

I

Un bà dzor de jeuillet, lo dzor de la Revenna,
De Veulla dze m'en parto a l'arba di matin,
In porten aprë mè: salan, pan blan, fontenna,
E tsecca de ci cllier que se feit din la tenna,
Pe me bletti lo pot lon de mon tsemin.
A Saint-Pierre dzi prei lo tsemin que meinàve
Su di coutë de Vertozan.
Dzà pe Saint-Nicolà lo mondo s'apprestàve
Come cen se feit tseut le-s-an.
I.é, qui d'un coutë crie et qui de l'âtro braille
Hoé! hoé, parten-nà, le-s-ami?
Di vatse se vat fére euna groussa bataille;
Maque degadzen-nò, l'est l'aoura de parti.

II

Bientou se sent lo flà di violette neissente,
Qu'imboumon l'air frëque de Vertozan:
Bientou dze sento dzà que le béque pouegnente
Repondon a bë-tor, i sublo di s-arpian.
Pe le prà, tseut in fleur, qu'un eigue pura arrouse
Dèsot l'erba catsà tsante lo greseillon
Di boueisson i sapin lo rossegnon se pouse,
Et regale i passen se pi belle tsanson.
De llioen se veit qu'i Breuil embouon leur vatse pleine,
Que bedzolàvon dzà, senten lo tsaat di dzor;
Dze traverso lo plan, yaou dzouëre se promeine,
Et que partadze in baillen de détor.

III

In arreuven i Breuil dz'i vu, come euna fëta;
Tot lo mondo achouedzà di pià tanque a la tëta.
Le femalle, ci dzor, l'ayan de dzen pitset,
Fran come voulon leur; cen restàve se ret!
(…)

(*Jean-Baptiste Cerlogne)*

Cow fighting in Vertosan

I

A beautiful day in July, the Revenna day,
I left Aosta before first morning light,
bringing with me salame, white bread, fontina cheese,
And a little bit of that juice that is made in the wine vat,
To wet my lips along my way.
In San Pierre I've taken the path
leading up to Vertozan.
In Saint-Nicolas people were already getting ready
As they're used to do every year.
There, someone was calling, someone shouting
Hoé! Hoé let's go, friends!?
A big cow battle is brewing,
Come on, it's time to go.

II

We were already smelling the scent of newborn violets,
which are perfuming Vertozan fresh air:
Soon I can hear that the sharp mountain peaks
All around are replying to herdsman whistles.
In the green pasture, sprinkled by pure water,
A cricket is singing hidden beneath the grass.
From the bush to the fit tree a robin flew,
Giving passers its best songs as a present.
From a distance we saw that in Breuil
the sated cows are sending back into the barn
running, feeling the heat of the day.
Through the tableland where the stream wanders around
dividing it with its curves

III

Entering in Breuil I saw, as in a feast day,
Everybody well-dressed from their head to their feet
Women that day were wearing nice laces
So stiff as they want!
(…)

(Italian translation by Elena Tessitore)

GENERAL INDEX

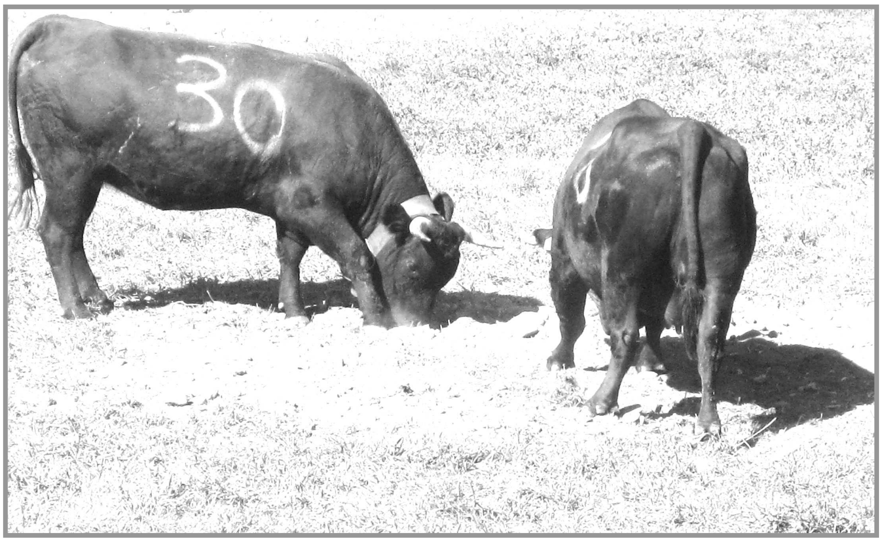
General Abstract

Fighting ability is a well-known attitude in Valdostana cattle (i.e., Aosta Chestnut and Aosta Black Pied cattle), due to the strong belligerency that cows exhibit at pasture, when unfamiliar animals met and new hierarchies for the access to resource have to be established. This peculiar attitude revives during the traditional tournaments of Batailles de Reines, annually performed by cows in the Aosta Valley. Annual competition consists of 20 eliminatory tournaments and a final challenge, where only the two autochthonous breeds are allowed to take part in the battles. Using data coming from the battles, fighting ability has been investigated aiming to assess both the behavioural and genetic aspects. Four steps have been followed, aiming to look at fighting ability under different but complementary point of views and to shed light on different concerns. At first, data from 4 tournaments undertaken in 2009 have been video recorded and analysed (i.e., a total of 168 fights) in order to depict both the dynamics of agonistic interaction among cows and what factors may affect the shape and the outcome of a conflict. A suitable phenotypic score for fighting ability (i.e., Placement score) was developed in a second study using data from 6 years of battles (i.e., 2001-2006, approximately 16,000 records belonging to about 6,000 cows). This in order to build a genetic model able to investigate the variance components of fighting ability and to quantify genetic parameters. Additionally (i.e., third study), the analyses included the incidence of conspecifics in the genetic estimates of fighting ability, considering the introduction of the opponent either within the phenotype itself or directly in the model as indirect genetic effect (IGEs). As alternative analysis, the contribution of conspecifics has been retained directly into the phenotype, implementing the previous score into a Competitive Placement Score, thus applied into analogous genetic models. As further step, the effect of another force (i.e., inbreeding) and its relationship with the genetic values for fighting ability has been investigated in Aosta Chestnut and Aosta Black Pied cattle. Population data coming from the entire pedigree, as well as all available information on 9 years of battles (i.e., about 24,000 records of over 8,200 participants) permitted to assign individual inbreeding coefficients to cows. Thus, the incidence of inbreeding into genetic models for fighting ability was studied, as well the possible relation among genetic merits and inbreeding. Results obtained from this study indicate that fighting battles among cows seem to follow the typical dynamic of an escalated

contest, with a cumulative assessment of the two contenders. Moreover, age, weight, and, most of all, prior experiences of the previous battles play a role in determining the outline and the intensity of the conflict. In addition, the genetic component of fighting ability results of main importance in affecting the outcome of the encounters. As well, weight, age, herd and tournament revealed as significant factor in the investigation of phenotypic variance, and, together with a direct additive and a permanent environmental, they have allowed the estimates of “non zero” genetic parameters. Heritability of fighting ability showed a level of about 0.08 when evaluated using a classical quantitative model, whereas the introduction of indirect genetic effects drove heritability estimates to levels of approximately 0.11. Moreover, including indirect genetic components showed a better general model fitting, whereas classical quantitative models taking into account ~~of t~~he opponent within the score exhibited the worst fitting. Reasoning on the different variance components that can be accounted into the model (i.e., either direct or indirect and due to conspecifics, as well as related to the permanent environment), models that included the opponent only as genetic effect have provided better estimates. Including the kinship as classes of inbreeding coefficient within the genetic models, heritability estimates undergo some small variations under classical quantitative models (i.e., +0.02) whereas models with IGEs did not experienced any shift due to inbreeding. However, the genetic values for fighting ability resulted ~~as~~ reduced in correspondence to increasing levels of inbreeding, as shown by the negative slope of the linear regression analysis performed on lineages of fighting cows with increasing inbreeding levels. As well as inbreeding shows a positive trend over years due to selection, it is interesting to note that, despite the incidence of inbreeding depression and a lack in planned selective programs, also fighting ability reveal a positive increase in mean breeding values (about 2-3% of gain/year). Thus, models including indirect genetic effects are the most appropriate tool in investigating social traits, and the study of fighting ability in Valdostana cattle may provide some interesting suggestions for the analysis of social traits in the area of animal breeding.

Riassunto generale

Le razze Valdostana Castana e Pezzata nera tradizionalmente si contraddistinguono per una spiccata belligeranza che emerge al momento del pascolo, quando gli animali provenienti da mandrie diverse si incontrano e combattono per definire nuove gerarchie sociali. Allo scopo di riproporre tale comportamento ad un pubblico più vasto, gli allevatori valdostani organizzano da secoli una caratteristica competizione che prende il nome di "*Batailles de reines*" e vede annualmente migliaia di esemplari contendersi il titolo di "Regina dell'anno". Annualmente, la manifestazione consiste in 20 giornate di eliminatorie ed in un torneo finale a cui è consentito prendere parte soltanto alle bovine provenienti dalla Valle d'Aosta. I risultati dei combattimenti disputati nel corso degli anni sono divenuti materia di ricerca nel presente lavoro di tesi, allo scopo di studiare alcuni aspetti, sia genetici che comportamentali, dell'attitudine al combattimento nelle bovine valdostane. Il carattere in questione è stato studiato seguendo 4 passaggi successivi, condotti con metodologie diverse e volti a mettere in luce aspetti differenti del comportamento combattivo. La prima analisi si è focalizzata sulla dinamica delle interazioni agonistiche tra bovine combattenti, preoccupandosi di capire quali fattori possano incidere nel tipo di combattimento che viene espresso e sul suo eventuale esito. Allo scopo, sono stati considerati 168 combattimenti registrati mediante videocamera nel corso di quattro tornei svoltisi nella stagione 2009. Il dataset per le analisi è stato largamente ampliato nello studio successivo, allargato ai dati raccolti nel corso di sei anni di competizioni (dal 2001 al 2006, circa 16.000 record appartenenti a 6.000 esemplari), e volto a delineare un punteggio fenotipico (Placement Score) ben rappresentativo delle performance dei partecipanti. Tale punteggio è stato quindi utilizzato come variabile dipendente in un modello genetico volto a stimare le componenti di varianza ed i parametri genetici per l'attitudine al combattimento. Tale punteggio è stato quindi inserito come fenotipo in un modello genetico volto a stimare le componenti di varianza e i parametri genetici inerenti al carattere studiato. Un'ulteriore analisi (terzo passaggio), si è invece focalizzata sullo studio degli effetti genetici indiretti (IGEs) dovuti all'incidenza dei partner sociali nel fenotipo dell'individuo. Quale criterio di indagine, si è provveduto a confrontare modelli genetici privi dell'effetto dei conpecifici (i.e., membri della stessa specie), con modelli comprendenti invece l'avversario, alternativamente introdotto nel fenotipo (Competitive Placement Score) e nel modello genetico. Quale quarto e finale passaggio, è stato condotto uno studio di popolazione sul livello di inbreeding nelle due razze studiate, in grado di stimare coefficienti di parentela individuali. Tali coefficienti sono stati quindi inseriti nei modelli genetici (descritti in precedenza) allo scopo di determinare l'incidenza dell'inbreeding sulle stime dei parametri genetici per la combattività, nonché sul valore genetico degli individui consanguinei. Per quest'analisi si è reso disponibile un dataset più ampio, comprendente un ammontare di 24,000 record relativi ad oltre 8,200 esemplari. I risultati ottenuti a seguito di tutte le analisi condotte, dimostrano che i combattimenti tra bovine seguono le tipiche dinamiche della lotta scalata, costituita da valutazioni successive degli avversari con esibizioni ad intensità crescente. Quali risultano fattori chiave nello delineare l'esito dei conflitti e le dinamiche in cui essi si svolgono sono emersi l'età dei contendenti, il loro peso, e, soprattutto, le precedenti esperienze di combattimento. Analogamente, anche la componente genetica della combattività rivestire un ruolo significativo nell'influenzare l'esito dei conflitti. Le analisi statistiche e genetiche condotte su tale carattere hanno permesso di riconoscere come significativi fattori quali il peso, l'età, l'azienda e il torneo dell'esemplare, come pure le componenti genetica indiretta e ambientale. Le analisi condotte sui modelli quantitativi classici hanno permesso di stimare un'ereditabilità per l'attitudine al combattimento dell'8%, mentre le analisi effettuate sui modelli con effetti genetici indiretti hanno riportato valori di ereditabilità dell'11%. Confrontando le due tipologie di modelli, è emerso come l'inclusione degli effetti genetici indiretti porti a valori migliori nelle stime. Tra i vari modelli comprendenti le componenti indirette considerati negli studi, quello il più affidabile risulta includere gli effetti indiretti solo in termini di componente genetica additiva e non ambientale. L'introduzione del coefficiente di parentela nei modelli genetici, sia essi classici che comprendenti effetti indiretti, comporta delle variazioni soltanto lievi nelle stime dell'ereditabilità, dell'ordine del 2% modelli classici, e addirittura non percettibili negli altri. I valori genetici per la combattività sembrano comunque risentire negativamente dell'effetto dell'inbreeding, come suggerito dalla pendenza negativa della retta di regressione lineare ricavata analizzando genealogie di consanguinei con livelli di inbreeding crescenti. È infine interessante notare come, nonostante la mancanza di un'opera selettiva pianificata rivolta al miglioramento del carattere, l'attitudine al combattimento risulti comunque aumentare nel tempo, rivelando un incremento nei valori genetici del 2-3% annuo. Da questa, e dalle precedenti considerazioni effettuate per le precedenti analisi, è possibile concludere che l'attitudine al combattimento nella razza Valdostana può offrire al miglioramento genetico degli spunti di riflessione interessanti per l’analisi dei comportamenti e dei caratteri sociali.



Introduction

Living in social groups leads members to compete for accessing essential resources (Pusey and Packer, 1997, West-Eberhard, 1979). In such situations, how an individual behaves mainly depends on the opponent it has to face (Maynard Smiths, 1982). Measuring the inheritance of expressed traits needs to account for the interactions with conspecifics (Bleakley and Brodie III, 2009). Artificial selection in livestock provides an excellent framework for the operative investigation of some important dynamics of social living and related genetic incidences (Wade et al., 2010). The present work aims to review the different approaches proposed in past decades for investigating social behaviour, in order to analyse recent issues on fighting ability in cattle that constitute the chapters of this PhD thesis as well as a good example of investigation of a social trait following the suggestions of knowledge arisen in the course of the time.

PART 1 – FROM THEORY OF GAMES TO INDIRECT GENETIC EFFECTS: A JOURNEY ACROSS SOCIAL BEHAVIOUR, ANIMAL CONFLICT, AND THEIR GENETIC CONCERNS.

1.1 - The golden age of social traits

The genetics of social traits is one of the hottest topics in genetics. The strong interdependence existing among interacting conspecifics (i.e., members of the same species) may introduce an appreciable difficulty in genetic investigations when realised that environment, agent of selection, could be at the same time the genetic pool on which selection acts (Moore et al., 1997; Bleakley and Brodie III, 2009). The powerful tools of quantitative genetics (Boake et al., 2002; Kruuk, 2004) and the recent issues of "interacting phenotypes" and "indirect genetic effects" (Moore et al., 1997; Bijma et al., 2007a) have succeeded in providing a reliable framework for understanding the genetic components of social traits (Bleakley and Brodie III, 2009). In recent years, animal breeding practices have found out appreciable benefits when social components have been accounted (Bijma et al., 2007b; Van Vleck et al., 2007; Chen et al., 2008). The first genetic evaluations on social behaviours as predator avoidance (Bleakley and Brodie III, 2009) and sexual behaviour (Moore et al., 2001) have been nowadays carried out also in natural conditions. A noticeable experiment on aggressive behaviour was undertaken with California deer mouse (*Peromyscus maniculatus;* Wilson et al., 2009). In order to estimate genetic components of some behavioural patterns occurring in the course of a conflict, a series of aggression tests within a neutral arena has been performed among pairs of conspecifics. As common fixed effects able to explain part of phenotypic variance, the sex of the opponents, their order of arrival in the arena and the number of test repetitions were included in the models. Both the dynamics of agonistic interactions among conspecifics (invoking a series of behaviours with diverse intensities) and such mentioned factors affecting the phenotypes, have stirred up principles and concerns belonging to the animal conflict models developed over decades within the framework of game theory. As time went by, two main approaches have been developed around selection processes working on multivariate sets of traits: optimality theory models (Parker and Maynard Smith, 1990), that include game theory models (Maynard Smith, 1982), and quantitative genetics models (Arnold, 1994). Perceived as opposing for long time (Charlesworth, 1990; Roff, 1994), in the last decades the two approaches have been reviewed and compared finding out similarities and a possible synthesis (Moore and Boake, 1994; Pigliucci and Schlichting, 1997). The fresh effort arising from quantitative genetics of social traits may highlight how much more complete and powerful a holistic approach accounting for inputs coming from such different backgrounds can be.

1.2 - How to solve a conflict: what optimality approach and game theory can teach us

Built up from suggestions of Darwin (1859) and Tinbergen (1963), optimality approaches primarily aim to answer “why animals behave the way they do” (Moore and Boake, 1994). Therefore, they firstly ask what sets of individual conditions and behavioural strategies are favoured by selection and are able to maximize the fitness (Moore and Boake, 1994). Thus, a range of alternative strategies are assessed and tested taking into account accurate biometric measures and biological constraints (see Parker and Smith, 1990 for a review). Hence a model is built, in order to describe the phenomenon and to be able to explain how different factors invoked will drive the response to selection. A good example of optimality model is illustrated in Elner and Hughes (1978): the shore crab (*Carcinus maenas*) chooses eating mussels of average size and not the biggest ones available. As a matter of fact, as the size of prey increases, opening the thicker shell becomes more and more difficult. Thus, the best solution is to choose a prey that is adequately rich (i.e., not too small), but also easy enough to manipulate. Authors tracked a curve predicting the energetic gain of the crab in relation to the size of the prey; experimental observations confirmed such predictions about size and decision making of crabs, and hence the built model of optimal foraging.

Optimality models have been largely and successfully applied to problems about foraging or other decision making processes of individuals. Parker and Maynard Smith (1990) distinguished a simple and a competitive optimization in relation to whether or not the behaviour of an individual depends on what other individuals do. Game theory (Waldegrave, 1713), that Lewontin (1961) and most of all Maynard Smith (1974) adapted to animals studies, revealed as a valid approach for describing social situations as foraging in groups (Giraldeau and Caraco, 2000), avoiding a predator (Brown et al., 1999), or fighting for the access to resources, either food (Schmitz and Baldassarre, 1992) or mates (Clutton-Brock and Albon, 1979). The first application of game theory in animal behaviour is provided by the Hawk-Dove Game (Maynard Smith and Price 1973), explaining how conflicts can resolve within a population which members always behave either as a hawk (i.e., they attack and fight

Table 1. Theories of assessment processes in animal conflict and hypothesis for winner-loser effects (after Jennings et al., 2005, Rutte et al., 2006, Arnott and Elwood, 2009).

Theory	**Explanation**	**Reference**
	Models of conflict assessment	
Pure Self Assessment or “own RHP-dependent persistence”	*Both contenders assess information only on the own fighting ability, and persist in the combat merely in relation to that.*	*Taylor and Elwood, 2003*
War of Attrition Without Assessment	The winner is the contender that persist longer; RHP asymmetries lead to a contest without any assessment of rival’ RHP	Parker and Rubenstein. 1981; Mesterton-Gibbons et al., 1996
Energetic War of Attrition	The main cost is the energy necessary to displace repeatedly	Payne and Pagel, 1996, 1997
Cumulative Assessment	*Costs of injuries are given by both opponent’s and own actions*	*Payne 1998*
Cumulative Assessment	A contender may achieve both information on the opponent and its own status in order to decide for escalation or retreat	Payne 1998
Partial Mutual Assessment	An animal is able to gather information about itself and the opponent, even if the last is weaker	Prenter et al., 2006
Mutual Assessment	*Decision making about contest through the assessment of opponent’ fighting ability in relation to their own*	*Maynard Smith and Parker, 1976*
Sequential Assessment	Differences between opponents RHP are assessed through a repetition of actions and signals of increasing intensity	Parker, 1974, Enquist and Leimar, 1983; Enquist et al., 1990
Asymmetric War of Attrition	Animal plays as “owner” or “intruder” in a territorial dispute, and the winner results individual persisting longer	Parker and Rubenstein 1981; Hammerstein and Parker 1982
“Mixed Assessment”	*Opponents can assess different tactics or vary their behaviour over time*	*Arnott and Elwood, 2009*
Different Assessment Among Contestants	When rivals play distinct roles in the conflict (i.e., owner/intruder) they can behave differently	Briffa and Elwood 2004
Switching Assessment	Use of diverse assessment mechanisms in different steps of the conflict	Morrel et al., 2005
Opponent Only Assessment	Only the fighting ability of the opponent is evaluate without perception of its own strenght	Rillich et al., 2007
	Hypotheses of winner-loser effects	
Self-Assessment hypothesis	Use of prior experience in order to estimate own fighting ability Whitehouse, 1997	
Social-cue hypothesis	Rivals use cues arisen from prior fighting experiences in order to assess asymmetries	Rutte et al., 2006
Strategic Use of the Past Outcome	Win may provide access to resource, while defeat may imply a depletion of energy reserves. These factors may increase the chance of winning or losing again	Parker, 1974
Constraints of Regulative Processes or Changes in RHP	Winner-loser effects are a by-product either of hormonal processes regulating agonistic behaviour or of increments in strength or body reserves	Rutte et al., 2006

RHP=Resource holding potential (Parker, 1974).

every time they meet a rival) or as a dove (i.e., they always displace without attacking and then eventually retreat). With the assignment of specific scores for winning and losing to each behavioural strategy, it is possible to predict within a population the amount of individuals behaving as doves or as hawks when a condition of competitive equilibrium is reached (evolutionary stable strategy or ESS; Maynard Smith and Price, 1973). This simple model of animal conflict is extremely powerful, simplicistic and effective, yet never realized in nature. However, several other models have been proposed over the years from this model, aiming to describe in the best possible way the dynamics of animal conflicts (see Arnott and Elwood 2009 for a review). A summary of the main theories about animal conflict is provided in Table 1. As a main point, real conflicts often invoke a sequence of non-physical actions behaved by the two contenders in order to assess reciprocal fighting ability (or resource holding power, RHP; Parker 1974) and thus to decide if falling in a physical struggle or not. Such ritualized behaviours and honest signalling are able to transmit reliable information about contenders strength (Zahavi 1975), permitting to perceive eventual reciprocal asymmetries (Parker and Rubenstein 1981). Several species (i.e., red deer *Cervus elaphus*, Clutton-Brock and Albon 1979; house finch *Carpodacus mexicanum*, Jonart et al. 2007) fight in such a way, described by Taylor and Elwood (2003) as “sequential assessment game” (SAG). The matter of animal conflict has been improved over years through both alternative models explaining the assessment of the individual and opponent RHP, and the new wave of inputs provided by the winner and loser effects (Hsu and Wolf, 1999). Factors such as recent or remote past experience, age, changing in physiological conditions, variations in social status -all unrelated with RHP- affect the outcome of a fight (see Hsu et al., 2006 for an exhaustive review). Both in terms of RHP assessments or winner-loser effects, such factors underline an adaptive value of behaviour, milestone of all optimality models and of related game theory approaches. The best fitted strategy, that models are able to predict, is the result of selective forces, trade offs among traits, and biological constraints (Gould and Lewontin, 1979; Parker and Maynard Smith, 1990; Roff, 1994).

Critiques about optimality models concerned their attempt to invoke evolutionary mechanisms as cause of the observed behavioural patterns without any investigation on such mechanisms (Roff 1994). Moreover, they are able to predict the equilibrium of maximum fitness for a population, but natural populations are often far from this ultimate condition. The defence of optimality approach performed by Moore and Boake (1994) explains that many behaviours have a direct effect on fitness, and the fitness consequences of a behaviour (so important in optimality concerns) are due to evolution, and not only mere adaptations. Moreover, under optimality models the genetic variations are assumed, and selection, driving a trait to one –or more- optima, works on such variation. Finally, the identification of possible factors affecting the phenotype is of primary importance both in optimality and in quantitative genetic estimations (Moore and Boake 1994). Thus, genetic models may thus arise from assumptions built up under optimality concerns.

1.3 - What does it mean to carry out a genetic investigation of behaviour

Genetic models assume that selection works within a generation on polygenic (i.e., quantitative) traits that could present either a continuous range of phenotypes or discrete phenotypes expressed through thresholds. Additionally, significant environmental influences may occur in increasing the number of potential phenotypes expressed. Under the classical quantitative genetic model (Fisher, 1918), a phenotype (z) mainly arises from the sum of environmental influences (e) and an additive genetic component (a) that will be transmitted between generations, as: $z = a + e$. The proportion of phenotypic variance due to additive effect of genes is known as heritability of a trait (h^2). This parameter quantifies the resemblance between parents and offspring, and also determines the rate of response to selection (Falconer, 1989; Maynard Smith, 1989).

Behaviour traits are arguably the most complex phenotypes to study, due to both a great number of genes involved with multiple interactions and to a significant environmental plasticity (Plomin, 1990). Moreover, non genetic factors (Plomin, 1990) and non additive genetic components (Meffert et al., 2002) are able to significantly influence such traits. As a consequence, behaviour shows a low level of heritability. If compared with other types of traits, behaviour shows a heritability lower than heritability of morphological, and physiological traits (i.e., muscularity or milk production, respectively). Nevertheless, life-history traits, as survival or the age at first parity, reveal heritabilities similar to behavioural ones. A recent meta-analysis of heritability values, estimated within all morphological, physiological, life-history and behavioural traits (Stirling et al., 2002), found out an average level of 0.31 for behavioural traits, with a standard error of 0.013. To investigate the genetics of animal behaviour through a quantitative approach, both assortative breeding in laboratory and studies either within families (i.e., on half sibs/full sibs offspring) or within inbred strains (Boake et al., 2002) have been carried out in different taxa. Stirling et al. (2002) reported a broad list of species in which the genetic components of some behavioural traits have been assessed. In addition, an overview of different behavioural heritabilities estimated via quantitative genetic models has been provided by Jensen et al. (2008) for livestock. The greatest example of selection at work, domestication has changed behaviour even before the traditional genetic improvement for morphological, productive traits (Plomin, 1990). Sometimes intentional matings have been performed, in other cases selection for morphological or productive traits has given unpredicted responses in correlated behaviours (i.e., Plusquellec and Boissou, 2001). Animal models developed for livestock breeding have been recently applied to natural populations (Kruuk et al., 2004), and covariance between relatives (Hill, 1971), maximum-likelihood estimations (Patterson and Thompson, 1971) and Bayesian inference (Sorensen and Gianola, 2002) have been recognized as milestones for all quantitative evaluations of genetic parameters (Thompson et al., 2005). A strict multivariate relationship between fitness and phenotype has been thus recognized and detected (Phillips and Arnold 1989), whereas the magnitude of genotype-environment interaction (GxE; Via and Lande 1985) has been analysed to describe how phenotypes may evolve under the action of selective forces. Quantitative genetics is also a valid tool in investigating social behaviours, such as fighting ability (Silva et al., 2006), dominance rank (Nol et al., 1996) and courtship (Boake and Konisberg 1998). On the other hand, some traditional breeding schemes applied to animals reared in pens or cages resulted as not so efficient or even inadequate for selective purposes (Muir, 2005). This lead to the development of alternative approaches as the models including indirect genetic effects (Bijma et al., 2007b).

1.4 – The social gene: social interactions and indirect genetic effects

While a genetic estimation of behaviour is difficult (Plomin, 1990), investigation of social behaviour is even more difficult (Moore et al., 1997). Actually, social partners play two roles at the same time: both as environment selecting a focal trait (i.e., the target individual) and as phenotype target of selection (i.e., conspecifics are individuals as well, thus interacting with other social partners). In such perspective, social traits have been recently appealed as “interacting phenotypes” (Moore et al., 1997). The classical quantitative genetic theory ($z = a + e$, see previous section; Fisher, 1918) was extended by Moore et al. (1997) separating the social partners effects from the rest of environmental components: $z_i = a_i + e_i + \psi_{ij}a'_j + \psi_{ij}e'_j$. Here, the “indirect genetic effects” (IGEs) a'_j represent the genetic part of the phenotype of individual i due to the contribution of interacting conspecifics (i.e., social partners) j. The magnitude and the direction of the interaction are expressed by ψ_{ij}: the path coefficient estimating how much a trait of the focal individual i may change after interaction with a trait of the opponent j (Moore et al., 1997). An alternative but equivalent (McGlothlin and Brodie III, 2009) approach for modelling social behaviour via quantitative genetics was originally developed by Griffing (1967) and further revisited by Muir and Schinckel (2002) and Bijma et al. (2007a). They have modelled the phenotype (z) of a target individual arises as the sum of both direct (D) and social (C) components, $z = a_D + e_D + a'_C + e'_C$. Thus, the genetic variance of the focal trait is partitioned into an additive part D belonging to the focal individual itself, and an associative part C coming from the contribution of all interacting conspecifics. Such "variance partitioning approach" permits considering the influence of the social partners both in terms of genetic and permanent environmental effect (e'_C). Moreover, it allows to investigate a social trait without defining a specific opponent trait as interacting phenotype (McGlothlin and Brodie, 2009) but considering conspecifics' impact as an "heritable environment" (Moore et al., 2002). These two approaches have been developed and applied over years to a wide range of theoretical or applied studies. Interacting phenotypes rise also from the school of "maternal effect theories" (Cheverud, 1984; Cheverud and Moore, 1994), aimed to explain the incidence of maternal environment at the early stages of life (Cheverud and Moore, 1994) or during parental care (Hunt and Simmons, 2002). As first, male "indicator traits" of a good gene quality (Andersson, 1994) are effective in explaining what an indirect genetic effect is (Wolf et al., 1997). As time went by, various social behaviours and evolutionary mechanisms have been developed following the principles of Moore's modeling. Thus, the genetics of social dominance has been assessed (Moore et al., 2002), and models investigating the genetic variation in "competition-dependent" traits after a conflict over resource have been later estimated (Wolf et al., 2008). Some important topics of sexual selection have been also detected, such as how male display can evolve (Petfield et al., 2005). Moreover, the sexual conflict (Moore and Pizzari, 2005) and the "lek paradox" (Miller and Moore, 2007) have been explained under the light of interacting phenotypes too, whereas the coevolution of male and female reproductive traits has been operatively disclosed in the experimental populations of *Drosophila pseudoobscura* (Snook et al., 2010). Laboratory trials also facilitated the estimation of the coefficient of interaction ψ_{ij} (Bleakley and Brodie III, 2009) and how it changes under the action of sexual selection (Chenoweth et al., 2010).

The “variance partitioned approach” of Bijma et al. (2007a) stemmed from the theories of “group selection” (Wynne-Edwards, 1963) and “multilevel selection” (Keller, 1999). These two theories were widely argued across time by evolutionary biologists, but are an interesting approach for animal breeding (Wade et al., 2010). Whereas natural selective forces necessarily act at individual level, assortative breeding for productive purposes may offer fixed groups of animals (i.e., reared in cages or pens) on which selective are carried out by human intervention. Theoretical studies performed either on simulated populations or on real data of livestock extended the theory considering the effect of group size (Hadfield and Wilson, 2007; Bijma, 2010) or kinship (Ellen et al., 2007; Cheng et al., 2009). Moreover, statistical tools (Arango et al., 2005; Muir, 2005) and suggestions for optimum designs (Bijma et al., 2010) have been provided to promote such kind of methods in management of social species. As time went by, operative studies on both livestock and model species in laboratory have been carried out applying either Moore et al. (1997) approach or Bijma et al. (2007a) issues. A plethora of traits, either interesting for just productive purposes (i.e., average daily gain or ADG) or arising for evolutionary animal behaviour concerns (i.e., mating time within reproduction) have been thus investigated and both indirect and direct genetic components have been successfully estimated (see Table 4 for examples). After years of parallel improvements almost without reciprocal feedbacks, the actual trend is to delineate similarities and differences among optimality theory and quantitative genetics, in order to unify the two approaches in a unique, powerful synthesis (McGlothin and Brodie III 2009).

1.5 –When game theory meets indirect genetic effects

This long journey across optimality, quantitative genetics and indirect genetic effects indicates the power that a multidisciplinary approach may have in investigating the behavioural dynamics, fitness consequences, genetic value of traits and selection processes. The theory of games (Maynard Smith, 1982) and the following theories about animal conflicts that have been developed over years (Table 1), offer reliable scenarios on which a contemporary action of direct and indirect components may play a role in the evolution or improvement of a trait. Assessments among opponents in their competition for resources (Maynard Smith and Parker, 1976) reveal an increment in intensity when opponents are of similar strength. Reduced asymmetries lead to longer and more intense conflicts both when there is a mutual assessment of opponents fighting ability (Sequential Assessment game, Parker 1974; see Table 1) or only a self evaluation of own capabilities occur (Self Assessment game, Taylor and Elwood, 2003; in Table 1). Thus, asymmetries in factors related to RHP (Parker, 1974) of contenders, as body size or weaponry (see Table 2) may trigger a response in terms of agonistic effort, and also can be used to make good predictions about the type of conflict. What's more, traits such as body size may influence the level of aggressiveness both in the terms of direct and indirect genetic effects, as the size of opponent may affect the aggressiveness of focal individual. In addition, focal individual's size may influence opponents' behaviour. Thus, a genetic investigation of aggressiveness has to take into account both the direct and the indirect genetic effect of size. Hence, size follows the dynamics of the first type of interacting phenotypes (Moore et al., 1997), since it affects another trait of the social partner but it is not influenced by other eventual traits of such partners. Moreover, assessment of asymmetries may follows the dynamics of an escalated conflict (Parker, 1974) where the decision about persisting in the fight or retreating mainly depends on the honest (i.e., reliable, true) signalling of the social partner (Zahavi, 1975; Enquist and Leimar 1983; see also Table 1). Thus, submissive or agonistic postures of the rival may lead the response of the focal individual, and vice versa: such behavioural patterns of submission or dominance are traits that both influence another trait of the conspecific and are influenced by such target trait

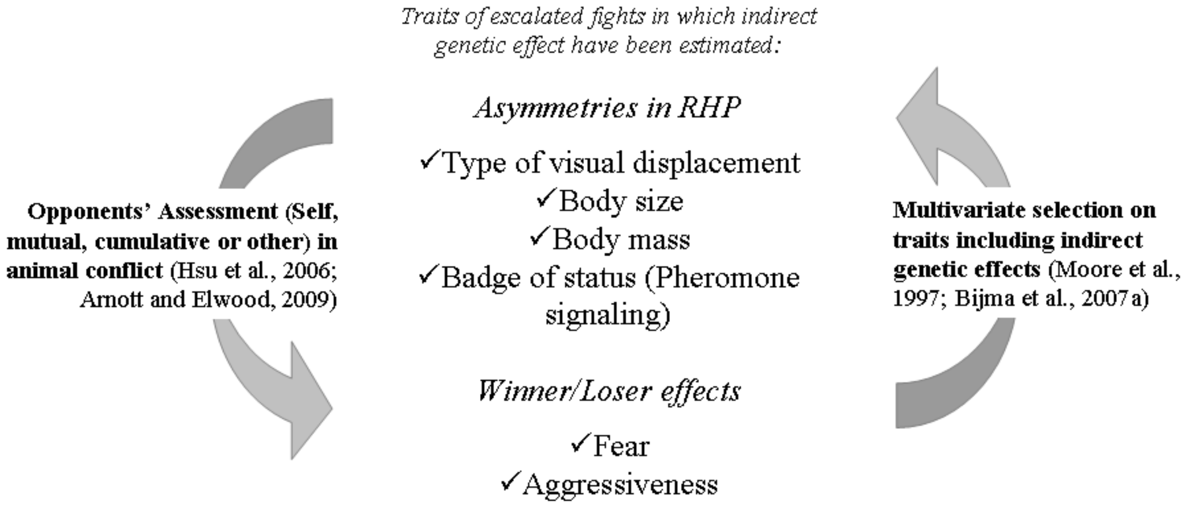
Table 2. Asymmetries and badges invoked in animal conflict (Modified from Hsu et al., 2006, Arnott and Elwood, 2009).

Asymmetries/Badges	
RHP[1]	*Winner/Loser Effect*
Acoustic signals	Resource values
Body mass	Physiological state
Body size	Chemical cues
Genetic Value	Recent experiences
Weaponry	Past experiences
Badges of status	Audience
Energy reserve	Age
Sex	Fighting tactic

[1] RHP=Resource holding potential (Parker, 1974).

at the same time. That's the second type of Moore et al. (1997) interacting phenotypes, already documented in nature when the social dominance in cockroach has been investigated (Moore et al., 2002). Here, the genetic components of pheromone displays of dominance have been assessed as indirect genetic effect. What's more, pheromone levels used in contests are also honest signals of the RHP of individuals. Figure 1 depicts the contemporary contribution of game theories and IGEs models in drawing the dynamics of animal conflicts. Meeting point and background for both issues are asymmetries in fighting ability existing among contenders and the incidence of eventual winner loser effects (Hsu et al., 1999). All the traits reported in pictures have been estimated in their indirect genetic components, as a comparison with Table 3 reveals. For example, the study on California deer mice reported by Wilson et al. (2009), found out indirect genetic values for five traits related to the aggressiveness that the

Figure 1. Traits involved in challenge assessments as influenced by indirect genetic effects. Table 2 and 3 reports traits that have been already analysed with IGEs.



RHP=Resource holding potential (Parker, 1974).

Table 3. Overview of traits studied with the approaches of indirect genetic effects.

Trait	**Specie**	**Reference**
Behaviour		
Activity (various types)	*Drosophila melanogaster*	Higgins et al., 2005
Aggressiveness	Pig (*Sus scrofa*)	Lovendahl et al., 2005
	Deer mouse (*Peromyscus maniculatus sonorensis*)	Wilson et al., 2009
Antipredator behaviour	Guppy (*Poecilia reticulata*)	Bleakley and Brodie III, 2009
Breeding time	Red-billed gull (*Larus novaehollandiae scopulinus*)	Teplitsky et al., 2010
Chemical display	*Drosophila melanogaster*	Kent et al., 2008
Copulation duration	*Drosophila pseudoobscura*	Bacigalupe et al., 2008
Courtship	Housefly (*Musca domestica*)	Meffert et al., 2002
Fear	Mink (*Mustela vison*)	Berg et al., 2002
Fighting ability	Cattle (*Bos bovis*)	Sartori and Mantovani, 2010
Maternal ability	Pig (*Sus scrofa*)	Lovendahl et al., 2005
Mating speed	*Drosophila pseudoobscura*	Bacigalupe et al., 2008
Sexual display	*Drosophila serrata*	Chenoweth et al., 2010
Social dominance	Cockroach (*Nauphoeta cinerea*)	Moore et al., 2002
	Human (*Homo sapiens sapiens*)	Hawley, 1999
Life-history		
Survival	Chicken (*Gallus gallus domesticus*)	Ellen et al., 2007
		Bijma et al., 2007
	Lamb (*Ovis aries*)	Everett-Hincks et al., 2002
Morphology		
ADG (average daily gain)	Cattle (*Bos bovis*)	Van Vleck et al., 2007
	Pig (*Sus scrofa*)	Cassady and Van Vleck, 2005
		Arango et al., 2005
	(swine)	Chen et al., 2008
	(boars)	Chen et al., 2009
Biometric meas. (length, area)	Mussel (*Mytilus galloprovincialis*)	Brichette et al., 2001
Body length	Atlantic cod (*Gadus morhua*)	Monsen et al., 2010
Body size	*Drosophila melanogaster*	Wolf, 2003
	Dung beetle (*Onthophagus taurus*)	Hunt and Simmons, 2002
Body weight	Atlantic cod (*Gadus morhua*)	Monsen et al., 2010
	Chicks (*Gallus gallus domesticus*)	Van der Waaij et al., 2010
	Japanese quail (*Coturnix japonica*)	Muir, 2005
		Muir and Schinckel, 2002
Hydrocarbons of wing	*Drosophila bunnanda*	McGuigan and Blows, 2010
	Drosophila serrata	Petfield et al., 2005
Female reproductive tract morphology	*Drosophila pseudoobscura*	Snook et al., 2010
Fattening traits	Pig (*Sus scrofa*)	Bergsma et al., 2008
Physiology		
Egg production	Chicks (*Gallus gallus domesticus*)	Muir, 1996
Embrio viability	Australian field cricket (*Teleogryllus oceanicus*)	Garcia-Gonzalez and Simmons, 2007
Sperm number and quality	Drosophila *pseudoobscura*	Snook et al., 2010

interacting competitors express. As literature on winner loser effects explain (Hsu et al., 1999; see also Table 1), the level of aggressiveness of a competitor may influence the grade of aggressiveness of the antagonist. That is consistent with the third and the last type of interacting phenotype described by Moore et al. (1997), where a focal individual's trait affecting the same trait of social partner (i.e., aggressiveness) is also influenced by such conspecific's trait. As Moore et al. (1997) consequently observed, social dynamics generally involve a series of behavioural patterns and morphological cues that may be better explained in terms of multivariate evolution. An interesting and pioneer example of such approach is provided by works on "activity" (i.e., sum of different behaviours) in *Drosophila melanogaster* (Higgins et al., 2005; see also Table 3).

The presence of interacting phenotypes may be found out under all the types of animal conflicts (Arnott and Elwood, 2009; Table 1); for example, sequential assessment game approach invokes a continuous evaluation of social partner with the aim to decide what is the best behaviour to perform in such situation. Even if the assessment is based only on the perception of own strength (i.e., self assessment), the final defeat of one contender necessarily affects further actions of both competitors. Indirect genetic effects can also be used to study how traits may change in response to a situation of intense social interactions (i.e., Bergsma et al., 2008). In such perspective, studies on social genetic components in livestock have aimed to detect how the intensity of competition may affect important morphological traits such as body weight (e.g., Monsen et al., 2010; but see also Table 3). Thus, body weight of rivals can be under the effect of indirect components' action and an asymmetry affecting the shape of a conflict at the same time. The synergy of different issues is thus revealed, as the power of a multidisciplinary approach in investigating the dynamics of social life.

1.6 – Behaviour & population genetics: mutation, drift, inbreeding and what they concern

As well as the other types of traits, behaviour that is realised in one environment is the result of a sum of different evolutionary forces contemporarily acting on traits. The genetic structure of a population and the phenotypes that are express are thus a consequence of the environment in which they are (i.e., Ridley, 2004). Under optimal conditions, a population is expected to exhibit alleles frequencies consistent with the relative fitness of traits. But, the optimal conditions in one environment may be not the best in another (Wright, 1984) and also, environment may change over time, after catastrophic events or simply cyclical changes in conditions (i.e., *Geospiza fortis*, Gibbs and Grant, 1987; *Lepus americanus,* Krebs et al, 1995). As also important, organisms themselves change over time (i.e., growth), and consequently, their internal environment changes as well. Thus, a trait is the result of a sum of simultaneous external and internal inputs that the different factors contemporarily acting exert. Four forces are recognized as main factors in affecting alleles frequencies (Hartl and Clark, 2007); mutation (Kimura, 1979), migration (Hanski and Gilpin, 1997), selection (Darwin, 1859) and genetic drift (Fisher, 1922). Two of these factor are able to enhance the genetic variance, either through the creation of new allelic variants (i.e., mutation), or when individuals (and genes) move from a population to another (i.e., migration or gene flow). Thanks to these main mechanisms and some important others (i.e., heterozygous advantage, Kalmus, 1945; genetic capture hypothesis, Birkhaead et al., 2006) genetic variability is maintained within populations. In opposition, natural selection (as well as sexual and artificial selection) and genetic drift act reducing genetic variability. As implied in the previous paragraphs, selection is the environmental pressure that favour some genes combinations due to the fact that organisms are diversely adapted to environment. As well, conspecifics (see previous paragraphs and Wolf et al., 1998) represent one relevant selective force acting on social traits. Another important evolutionary force, genetic drift is the random changing in allele frequencies over time due to stochastic processes (i.e., some alleles and not other are transmitted over generations by chance). This generally happens when the gene pool is dramatically shrunk, almost after events as bottlenecks (Wahl and Gerrish, 2001) or founder effects (Templeton, 1980), respectively due to strong reductions in population size or when a small number of individual establish a new population. Genetic drift is of primary important in small populations, that generally suffer in a lack of variability and may loose favourable allelic combinations generation by generation by chance. Reduction in population size may also lead to another important phenomenon: the increment of inbreeding within population. Inbreeding is the process of non random mating among relatives, thus traducing in increments of heterozigosity (Jacquard, 1975). Keller and Waller (2002) well described inbreeding as: i) the quantity of ancestry shared by the parents of an inbred individual, ii) the degree of relatedness among mates as respect to two mates chosen from population by chance, and iii) the mean inbreeding coefficient in subpopulations under random mating, under population subdivision.

The consequence of inbreeding is a reduction in fitness and in overall performances of traits (i.e., inbreeding depression) due to the exposure of deleterious combinations of alleles at phenotype level (i.e., dominance hypothesis), and also due to a loss in fit when selective pressures aware heterozygotes (overdominance hyphothesis). Inbreeding depression firstly affects traits strictly related to fitness (DeRose and Roff, 1999), as life-history features, such as longevity or survival. Interesting examples of inbreeding depression have been observed among livestock, as well as in laboratory and field (e.g., Keller, 1998; Slate et al., 2000). Negative effects of inbreeding have been also found out in other framework, such as in physiological traits (i.e., milk production in cattle; Miglior and Burnside, 1995; adaptation to harsh conditions; Burrow et al., 1998) and morphology (i.e., growth rate in livestock; McParland et al., 2008). Furthermore, inbreeding has revealed its effects also on behaviour, moreover on traits related to fitness as male courtship, or female choice (Ala-Honkola et al., 2009; Bohlund et al., 2010). Sexual selection may be nasty with inbred pretenders, that exhibit in less-quality songs (Aspi, 2000) or visual parades (Mariette et al., 2006) not appreciate by the possible partners. It is interesting to note that inbreeding depression is more effective on social behaviours, probably due to the immediate feedback among the less-quality features of an inbred individual and the refusal of a sexual partner. After the lack of mating during breeding season, individual fitness falls to zero. Other social behaviours, such as fighting ability and aggressiveness, have revealed to suffer inbreeding, such as both field and laboratory studies attested (e.g., Hoffman et al., 2004; Välimäki et al., 2007). Conversely, influence of inbreeding on traits not related to social context, as individual temperament, has revealed sometimes such as significant and sometimes as not (McParland et al., 2007; Dubcovà et al, 2009, respectively).

Among wild species, the risk of inbreeding is avoided mainly by behavioural mechanisms (Pusey and Wolf, 1996), as brood dispersal (resource competition hypothesis; Greenwood, 1980). Hence, behaviour may be the target of negative inbreeding effects (i.e., inbreeding depression) or, at the same time, the resource for avoiding inbreeding. For example, under the resource competition hypothesis, after a fight the defeated contenders have to leave territory and disperse. Thus, they avoid to mate with close relatives (e.g., *Canis simiensis*; Randall et al., 2007). Inbreeding may also affect the genetic value of behaviours (as well as the other types of traits), changing the breeding values and also the levels of heritability (i.e., when variability decreases, such as when homozygosis is enhanced, heritability of a trait is reduced). Thus, when a genetic or ethological evaluation of a behaviour, especially a social behaviour has to be carried out, the occurrence of eventual relevant evolutionary forces, such as selection, genetic drift or inbreeding depression, has to be taken into account in reason to infer suitable considerations.

Concerning livestock, inbreeding avoidance through appropriate behaviours is not possible, due to breeding practices that overwhelm sexual selection occurring in nature and annul the possibility to perform any behavioural mechanisms of avoidance. However, farmers are nowadays used in adopting some breeding strategies (Weigel, 2001) taking into account for the relatedness of individuals. Restricting the number of select sires to animals with an appreciable level of heterozigosity (Goddard and Smith, 1990) , such as performing a selection within sublines, and subsequent crossing of lines (Smith and Quinton, 1993) are two of a plethora of actual strategies of inbreeding avoidance in animal breeding. The problems that have occurred in many years of strong selection have recently highlighted the importance, in breeding programs, to consider all the possible evolutionary forces, as selection as well as genetic drift or mutation, that constantly act on phenotypes.

1.7 – Selection at work: fat pigs, long-lived hens and fighting cattle

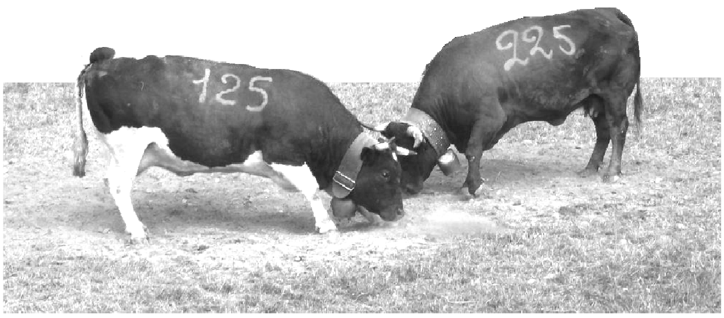
Genetic improvement of production in livestock provides an unique example of selective forces acting within a short temporal window. Breeding schemes have been implemented over years with new tools and concepts. Huge number of available records offers a powerful resource for quantitative genetic studies. In recent years, problems in the management of social species reared in groups of conspecifics, as hens or pigs has promoted the development of models accounting for indirect genetic effects (e.g., Muir, 2005; Bergsma et al., 2008). For example, the competition for feeding can lead serious injuries, with a consequent reduction in productive performance, i.e., lowered average daily gain (ADG) or even an increase in mortality. Dynamics of aggression has been well studied over time in both fowl (e.g., Estevez et al., 2002) and pigs (e.g., McGlone, 1985). Genetic evaluations performed via classical quantitative models (Fisher, 1918) have led to heritability estimates of 0.20 for aggressiveness in pigs (Turner et al., 2006) and about 0.14-0.18 in domestic fowls (see Jensen et al., 2008 for an overview). In recent years, the estimation of aggressiveness in pigs has been carried out also using the variance partitioning approach of Bijma et al. (2007b), resulting in total heritability estimates ranging from 0.03 to 0.25 for the trait. Breeding schemes accounting for IGEs have been developed over years for both pigs and fowls in order to reduce the aggressiveness within groups and to enhance the improvement of productive traits such as

ADG and other fattening traits in pigs (i.e., Bergsma et al., 2008, but see Table 3) and survival in chicken (Muir, 2005). Concerning cattle, indirect genetic effects for ADG have been assessed in Hereford bulls (Van Vleck et al., 2007), but this is the only case in literature up to now. Focussing on classical quantitative genetics, heritabilities of cattle behaviour have been estimated for traits such as temperament (Visscher and Goddard, 1995) or docility (Le Neindre et al., 1995). Fighting ability, in terms of aggressiveness and ferocity (Silva et al., 2006) or social dominance (Mantovani et al., 2007; Sartori and Mantovani, 2010), has been assessed in fighting breeds of bulls and cows (i.e., Ganado bravo, Hérens and Aosta Chestnut-Black Pied cattle). These breeds are involved in traditional cruel (Ganado bravo) or bloodless (Hérens and Aosta Chestnut-Black Pied cattle) tournaments that have centuries long tradition. Fighting performances recorded during such competitions have revealed a proficient tool for investigating fighting ability and related behaviours, suggesting also an intriguing scenario for carrying out studies of genetics of social traits.

PART 2 – SUBJECT OF THE STUDY

Aosta Chestnut and Aosta Black-Pied breeds are the two rustic cows of West-Alps involved in the traditional fighting tournaments promoted by farmers of Aosta region (Mantovani et al., 2007). Such exhibitions evoke the competitions for social dominance that naturally occurred on pasture at the beginning of grazing season. Cows from different herds that are joined at the same pasture engage agonistic interactions in order to assess a hierarchical order for the right over food resources. The spectacle of their fights, enhanced by a peculiar strong temperament, has led farmers to organize yearly traditional competitions well appreciated both by farmers and tourists. Concerning the breed, the origin of these cattle can be found out in the ancient *Bos brachyceros*, characterized by a wide head and recognized as an ancestor of some breeds in the alpine arc, such as the Heréns cattle of Switzerland (i.e., the Swiss fighting breed). Aosta Chestnut probably derives from a population of Heréns cattle that was introduced in Italy through mountain passes (Del Bo et al., 2001) and perhaps then crossed with local cattle. Aosta Black Pied is probably derived from Illyrian Celtic cattle of North Europe (FAO, 2011), but it may also be an indigenous breed. Both breeds (Aosta Chestnut and Aosta Black-Pied) are related to the other local breed of Aosta (i.e., Aosta Red Pied) and the Swiss breeds Heréns and Evolene, constituting a monophyletic group (i.e., with common ancestor) of local breeds (Del Bo et al., 2001). Aosta Chestnut and Aosta Black Pied are managed in the same herd book since 1985 (FAO, 2011). However, genetic improvement aims to preserve different and typical coat colorations of each variety. Population data provided by the national farmer organization of Valdostana breed (ANABoRaVa), managing the breed in collaboration with the Valle d'Aosta region and the regional association of breeders (AREV), indicate that in 2010, the Aosta Chestnut breed accounted for 11,958 cows, 147 bulls and 10,752 calves, for a total of 22,857 animals. On the other hand, data from the same year for Aosta Black Pied breed consisted of 1394 individuals, including 745 cows, 645 calves and only 4 bulls. Both breeds are not endangered (FAO, 2011), but the small number of individuals in Aosta Black Pied needs to be constantly monitored by local breed associations.

Both strains are selected for milk and meat, with a particular emphasis on milk, which is mainly used for the production of the Fontina cheese. In 2009 the average production, computed on both breeds together, were of 3,110 Kg, 3.45% and 3.44% for milk, fat and proteins, respectively (i.e., at the third lactation). Young bulls are evaluated through both performance and progeny test. At the end of the performance trial, their weight is approximately 300 Kg. Genetic improvement is carried out using an aggregate selection index in both sexes, including milk and muscularity evaluated at same relative weight. In the last two decades, an antagonism between milk production and fighting performance has led to disputes among the breeder association and farmers that bring their animals to the traditional battles. To conciliate the interests of both parts, the national breeder association (ANABoRaVa) requested Professor Roberto Mantovani, University of Padua, to undertake a genetic investigation of fighting ability in these varieties of cattle. Based on data collected at traditional tournaments over years, genetic evaluation started in 2007 and it has been carried out over the years till now, succeeding in assessing variance components of fighting ability and in computing related genetic indexes (Mantovani et al., 2007; Sartori and Mantovani, 2010). Nowadays, fighting ability has been included in breeding goals within the selection in addition to milk and muscularity. The evaluation of economic weights for the aggregate index is ongoing. When selection for the three characteristics will be realised, a new era of animal breeding will start.



PART 3 – AIMS OF THE THESIS

The present thesis arises from the collaboration between the University of Padua, Department of Animal Science, and the national breeder association of Valdostana cattle (ANABoRaVa). To generate genetic indexes for fighting ability (see Part 2 for detailed description) suitable for the genetic improvement of Aosta Chestnut and Aosta Black Pied cattle, a detailed genetic and behavioural investigation of the trait has been carried out between years 2008 and 2010.

The aim of the present thesis is to carry out an investigation of fighting ability using data coming from “Batailles de Reines”, the traditional tournaments that Aosta Chestnut and Aosta Black Pied breeds yearly engage. Moreover, some important considerations on the relations among genetic, behavioural, environmental and evolutionary components of fighting behaviour and of other social behaviours have been analysed and discussed.

The study has been structured as follows:

i) A behavioural analysis of the agonistic performances of Aosta Chestnut and Aosta Black Pied cattle. After quantifying the main behavioural pattern expressed by cows during the battles, the agonistic performances have been analysed and an ethogram (i.e., catalogue of behaviours) of the escalated conflict has been built. Statistical models have been implemented to find which factors could mainly affect the shape and the outcome of the contest.

ii) An evaluation of fighting ability has been carried out using the tools of classical quantitative genetics. As first, a suitable phenotypic expression of the trait has been achieved, followed by an investigation of variance components. Genetic parameters have been estimated and so the level of heritability has been assessed.

iii) The genetic model developed in the previous study has been implemented in order to account for the strength of interacting conspecifics. A comparison between a classical quantitative model including the competitor within the phenotype and a model accounting for the indirect genetic effects of conspecifics estimated using the “variance component approach” has been carried out (see Introduction, part 1.4), followed by a comparison among different genetic models accounting for genetic and permanent environmental genetic effects. Variance component estimates have been compared in order to quantify the magnitude of indirect effects when accounted in a genetic model for social traits.

iv) A population analysis on both Aosta Chestnut and Aosta Black Pied cattle has permitted to estimate the incidence of inbreeding within the two populations, quantified by inbreeding and average relatedness coefficients. Thus, the incidence of kinship on fighting ability has been evaluated, in order to assess any evidence of possible inbreeding depression on the behavioural trait.

The thesis ends with general conclusions in which a reflection on the behavioural and genetic evaluation of fighting ability is carried out. Quantitative genetics and optimality approaches applied in the study are evaluated in order to offer a possible synthesis and an example of a powerful tool for behavioural and genetic studies as well as for animal breeding practices.

Chapter 1

Running head: Agonistic behaviour in cows

DYNAMICS OF AGONISTIC BEHAVIOUR IN COWS USED FOR BLOODLESS COMBATS

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“The Resource Holding Potential (RHP) is a measure of
the absolute fighting ability of a given individual
Size, strength, weaponry and experience
all seem involved in RHP”
(Parker, 1974)

ABSTRACT - This study was aimed to analyze the dynamics of agonistic interactions occurring between cows that participate in traditional bloodless fighting competitions in the region of North West Alps, Italy. Specifically, the study aimed to build an ethogram (i.e., catalogue of behaviours), in order to investigate changes over time in conflicts, and to analyse factors affecting the outcome of a fight. An amount of 188 competitions was retained for statistical analyses. A first sub dataset, consisting in battles of three preliminary and the final tournaments kept in 2009, belonging to the 1st, 3rd and 5th encounter engaged was selected for the first part of analyses; altogether, 120 competitions. Only repeated wins belonging to 51 cows were retained and analyzed in order to obtain a transitional probability matrix of behaviours, and aiming to investigate the shape of the conflicts. Analysis of variance was performed with Mixed model for repeated measurements for the following variables: match duration, intensity, time spent on total behaviour of non agonistic interactions, exhibitions, and physical fights. Subsequently, in order to analyze factors affecting the outcome of a fight, a logistic regression analysis was carried out on all the 188 binary fighting results. A typical behaviour pattern of escalated conflict (i.e., defence of the resource, exhibition, and fighting) was obtained and described using the transition diagram of behaviours. Among factors affecting the shape (i.e., the structure, as the succession of different behaviours during an agonistic interaction) of the conflict both physical factors (weight), and aspects joined to previous experiences (age) showed a significant effect ($P<0.05$) on exhibitions (weight), on match intensity (weight and age) and on non agonistic behaviour (age). However, the most important factor affecting the shape of a match was the number of encounters. From the first to the following encounters there was a increase of intensity accompanied by a reduced frequency of exhibition behaviour and increased frequency of physical behaviour. The outcome of the match was not dependent by the order of arrival in the arena, but was influenced by difference weight and age differences between contenders ($P<0.05$). However, the greatest role in affecting the result of the battle was manifested with additive genetic effects for fighting ability, that showed a strong significant role ($P<0.001$) in influencing the probability of a win in a fight.

Key Words: agonistic behaviour, Resource holding power, behavioural dynamics, fighting, cattle

INTRODUCTION

Life in social groups is characterized by competition for limited resources resulting in a complex pattern of dominance hierarchies aimed to regulate access to resources (Pusey and Packer, 1997; Clutton-Brock et al., 2006). An agonistic interaction can be totally physical, not physical at all, or a mixture of displays and agonistic contacts (Maynard Smith and Price, 1973), depending on the value of resources, the cost of possible injuries, and on the kind of asymmetry between opponents. Before engaging into physical interactions, contenders generally assess an escalated fight (Parker, 1974). They evaluate the opponent’s fighting ability or the so called resource holding power (RHP) through signals expressed with increasing intensity (Maynard Smith and Harper, 2003; Manser et al., 2001). The outcome of a fight may depend on many factors (Huntingford and Turner, 1987; Jackson, 1991; Hsu and Wolf, 1999), such as previous fights engaged between dyads or the differences in aggressiveness due to the experience of agonistic interactions (Wong and Balshine, 2010). On the other hand, when contenders are close in rank, the duration of the struggle may increase in order to allow animals to acquire more reliable mutual information on the opponent’s strength (Enquist and Leimar, 1983). In the last decades, several studies have focussed on factors affecting the shape (i.e., the structure, as the sequence of behaviours during an interaction) of a conflict in social species, and some explicative models have been proposed (see Arnott and Elwood, 2009 for review), and confirmed by field data (Prenter *et al.*, 2006). Among domestic species, cattle (*Bos taurus*) represent an interesting subject for studying fighting, because they regulate the access to the natural resource at pasture through agonistic interactions of social dominance (Oberosler *et al.*, 1982). In this species, the main factors determining the outcome of a conflict have been already studied (Schein and Fohrman, 1955; Bouissou, 1972; Collis, 1976; Murphy and Duarte, 1990) and methods for assessing social dominance have been developed (Beilharz and Zeeb, 1982; Val-Laillet *et al.*, 2008). Moreover, recurring behavioural patterns exhibited during conflicts have been reported (Collis, 1976; Reinhardt *et al.*, 1986; Gibbons *et al.*, 2009). However, the dynamics of an escalated conflict among cattle have not yet been described. In this regard, the present study was aimed to analyse the dynamics of agonistic interactions that occur between cows of the Aosta Chestnut and Aosta Pie Black breeds that participate in traditional fighting competitions organized by farmers as a revival of the agonistic performances naturally occurring at pasture. Specific objectives of this study were: i) to outline the dynamics of escalated conflicts between cows and to define behavioural patterns; ii) to investigate how the structure and intensity of fights may vary over time during tournaments, and iii) to analyse factors affecting the outcome of a fight.

MATERIALS AND METHODS

Description of the subject and Data collection

Agonistic behaviour of cows was evaluated by observing battles performed between pairs of cows during the "Batailles de Reines": traditional competitions with centuries long tradition in Aosta Valley of Italian North-West Alps (Mantovani *et al.*, 2007). These competitions are a major folkloristic activity recurring in the Aosta region, as well as a remarkable source of attraction for tourists. Cow battles are not cruel, because animals perform the same type of behaviour as at pasture when hierarchical relationships are established. Battles are performed between pairs of pregnant milking cows of the Aosta Chestnut and Aosta Pie Black breeds. These two breeds are rustic autochthonous breeds of West Alps and possible direct descendant of primitive aurochs (*Bos primigenius*). Nowadays, traditional tournaments are yearly organized also in the French region Haute-Savoie and in Canton Valais (Switzerland), where fights are carried out with the Heréns cattle (Plusquellec and Bouissou, 2001; Plusquellec, 2001). A detailed description of "Batailles de Reines" has been already provided elsewhere by Sartori and Mantovani (2010). Yearly tournaments consist of 21 days with battles (20 eliminatory and a final competitions) beginning in the late March and ending in the middle of October. In order to introduce a setting similar to the natural environment, cows are leaded to fight in a grass arena with a diameter of 50 metres. Before going into the competition, cows are weighted, checked for milk production and pregnancy, then divided into three weight categories. A tournament schedule for each weight category is arranged dividing the participanting cows in pairs by chance. The competition consists of 4-7 contemporary knock-out battles where the loser has to leave the contest, and the winner gains the right to engage a new match against the winner of another competition, i.e. moving to an upper level of difficulty. Such a kind of challenge may consist of 4-7 levels of fights, depending on the tournament size.

Data on agonistic performances among cows were collected in the last four tournaments of “Batailles de Reines” in year 2009. Daily competitions occurred in the grass arenas at following locations: Gressan, Nus, Saint Christophe and Aosta (final match of the year).

Table 1. Descriptive statistics on the data of “Batailles de Reines” of the target four tournaments.

	Location				Weight (Kg)	Age (years)
	Gressan	Nus	S. Christophe	Aosta		
Cows	172	254	316	180	588±68	6.20±1.64
Cows in weight category 1	49	84	112	62	669±47	6.66±1.61
Cows in weight category 2	65	91	105	62	571±14	6.22±1.66
Cows in weight category 3	58	79	99	56	521±16	5.69±1.51

Table 1 reports some descriptive statistics concerning the focussed days of tournament. All the battles engaged were recorded using four different digital video cameras with miniDV, mini-DVD and SDHC cassettes as supports, for approximately 6-7 hours of recording per day. Thus, 883 of the all 922 battles were collected for subsequent analysis. Additional information on animal ID, individual weight at fight, herd and genealogical information were obtained from the farmer society that organize the tournaments (Amis des Batailles de Reines), the regional breeders Association of Aosta Valley (AREV) and the National Breeders Association of Valdostana breed (ANABoRaVa).

Behavioural observations

Video recorded data were analysed using Avidemux^{TM} and JWatcher^{TM} (Blumstein and Daniel, 2007) programs for the analysis of videos and behaviours, respectively. Behaviours were collected through a focal sampling and continuous recording (Altmann, 1974) of 120 battles chosen from the first, third and fifth encounters performed by cows, in order to get a balanced dataset (i.e., representative of the whole battle). An ethogram was built including all the behaviours observed in the course of the competitions and also taking into account information available from the literature (e.g., Collis, 1976; Gibbons *et al.*, 2009). Behaviours performed by the two contestants of a battle were always considered separately, in order to obtain cow specific ethograms. A score from 1 to 6 was assigned to each behaviour on the basis of agonistic intensity (Table 2). Target behaviours with the respective intensity score shown in parenthesis were:

- friendly (1): cow gently touches opponent with head or licks its muzzles;
- passive (2): cow stands in the arena without care for the opponent;
- defence of resource (3): cow rubs its muzzle in the earth and scrapes the ground, indicating its rights over the resource;
- vocalization (3): cow raises its nose up and bellows;
- visual display (3): cow stands in the front of the opponent and displaces (i.e., it communicates to the opponent its own strength through a physical exhibition) using the shoulders. Both contenders can also display at the same moment, standing up in order to assume a parallel orientations, with the heads in the same direction. As alternative they may take an anti-parallel orientation, turning their heads in opposite ways;
- looking in eyes (4): cow stands in the front of the opponent and stares into opponents' eyes. As a consequence, the rival takes immediately the same position;
- pushing (5): cow pushes the opponent with heads, leveraging on the ground with legs. Thus, the opponent does the same. As variation, a cow can push the body of the opponent with head, neck or own body. In such cases the opponent stands still, searching to contrast the attacker;
- vigorous clash (6): forceful conflict in which cow moves inside the arena and uses head, horns or the whole body to overpower the opponent. Sometimes a cow is capable to lift up the rival with the head.

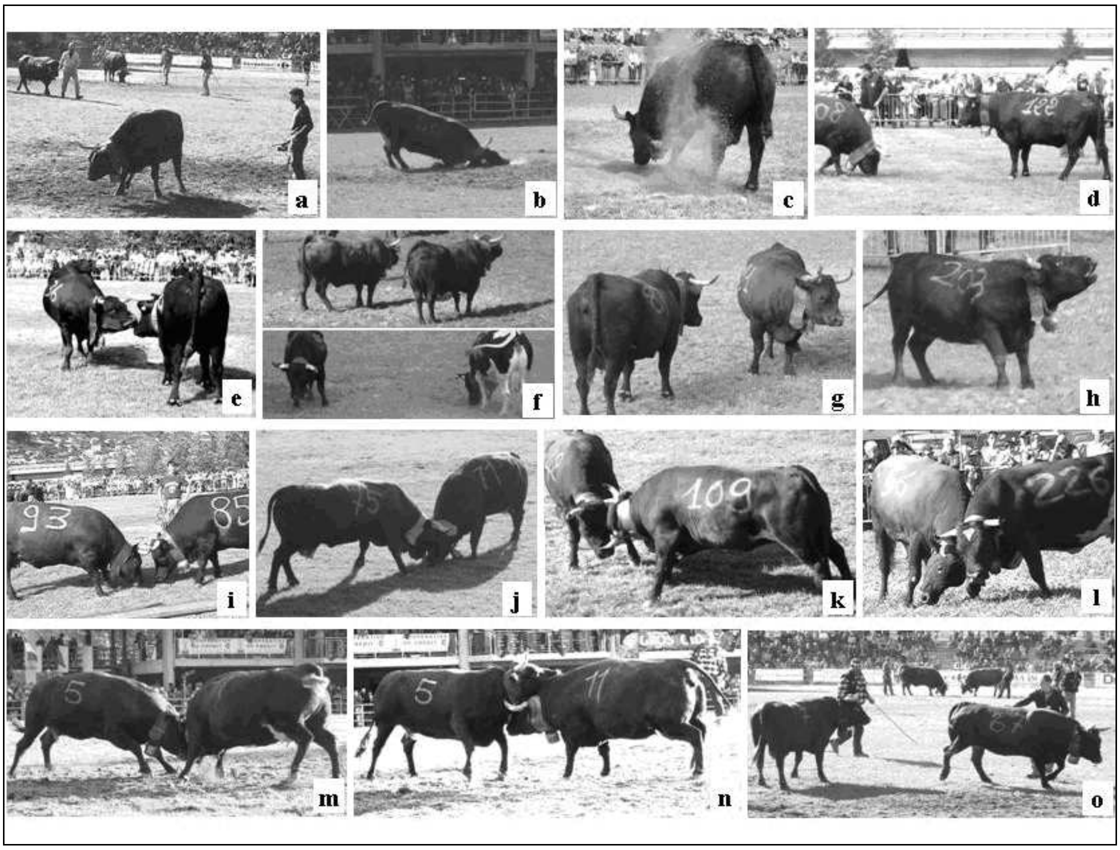
Duration of all behaviours were measured in seconds, and repeatitions in the course of a match were summed to obtain the total amount of time of time for each behaviour. An exhaustive scenario of all considered behaviours is reported in Figure 1.

Analysis of data

i) Diagram of behaviours

This analysis was carried out on 120 selected battles engaged by 145 participants and belonging to the first (n=48 battles), third (n=48 battles) and fifth (n=24 battles) round of

Figure 1. Examples of behavioural patterns$^{(\dagger)}$ performed by cows during the traditional "Batailles de Reines" with the type of behaviour in brackets.



$^{(\dagger)}$a. arrival of cows at the patch; b. rubbing the muzzle in the earth (defence); c. scraping the ground (defence); d. passive posture (not agonistic behaviour); e. friendly approach (not agonistic behaviour); f. reciprocal either parallel or anti-parallel orientation (visual display); g. threatening posture (visual display); h. vocalizations; i. looking in eyes; j. head-to-head (pushing); k. pushing with horns (pushing); l. pushing with neck (pushing); m. vigorous clash moving across the arena (vigorous clash); n. lifting up the opponent (vigorous clash); o. retreat of the loser and dominant posture of the winner.

matches from the four considered tournaments (Table 2). Once a winner of the first or third match round was observed, it was followed also in the subsequent matches in order to obtain repeated (2 or 3) observations per animal. The transition diagram with associated transitional probability matrix was built from the ethogram of 51 winners using the sequential analysis tool of JWatcher™ (Blumstein and Daniel, 2007). Values in the transitional probability matrix were obtained dividing the observed frequency for an event pair (e.g., vocalization and

Table 2. The percentage of observed behaviours (as respect to the total number) by the match rounds and the intensity score and type of interaction assigned to each behaviour.

	Match disputed			Intensity score	Type of interaction
	1st	3rd	5th		
Match considered (no.)	48	48	24	-	-
Behaviour:					
- friendly (%)	1.24	1.49	0.69	1	Non agonistic
- passive /(%)	7.40	6.07	1.76	2	Non agonistic
- defence of resource (%)	51.46	37.71	34.50	3	Exhibition
- visual display (%)	18.91	16.87	25.92	3	Exhibition
- vocalization (%)	0.82	1.42	1.90	3	Exhibition
- looking in eyes (%)	3.64	6.82	2.66	4	Exhibition
- pushing (%)	2.27	7.16	10.61	5	Physical fight
- vigorous clash (%)	14.26	22.46	21.96	6	Physical fight

looking into eyes) by the frequency of the first behaviour (Blumstein and Daniel, 2007). It has to be noted that a preliminary analysis of variance between losers and winners did not indicate any significant differences in behavioural variable measured for winners and for losers.

ii) Shape of the conflicts

The same 120 selected matches were used to analyze factors affecting the shape of a conflict. Data of the 51 winning cows were considered in order to retain at least the outcome of two matches per cow. The result of each battle was summarised with five variables:

i) the total duration of match, expressed in seconds;

ii) the overall intensity of match (I), computed as $\sum_{i=1}^{n}(s \cdot d) / \sum_{i=1}^{n} d$, where d is the duration of the i-th behaviour observed during the match, and s is the intensity score assigned to the behaviour (Table 2);

iii) the ratio between the duration of non agonistic interactions (Table 2) and the duration of the whole match (i.e., relative frequency of non agonistic interactions);

iv) the ratio between the duration of exhibitions (i.e., not physical contacts; Table 2) and the duration of whole match (i.e., relative frequency of exhibitions);

v) the ratio between the duration of physical fights throughout the match (Table 2) and the total combat length (i.e., relative frequency of physical fights).

Individual data were analysed using a mixed model (MIXED procedure, SAS Institute, 2004) for repeated measurements:

$$y_{ijk} = \mu + T_i + C_j + T_i*C_j + a_{k:ij} + NM_l + b_1*W_k + b_2*A_k + e_{ijk}$$

where y_{ijk} is the individual observation on the k-th cow, μ is mean (intercept), T is the i-th day of tournament (i = four levels), C the j-th weight category (j=three levels), T*C is the interaction among T and C, $a_{k:ij}$ is the individual random effect of a cow within T*C, ~N (0, σ^2_a), NM is the effect of the l-th match (l=3: 1st, 3rd or 5th match engaged) of a cow in a given tournament and category, b_1 and b_2 are respectively the regression coefficients of W_k and A_k, the individual covariates of difference in weight (Kg) and in age (years) that the target k-th individual had with the opponent, and e_{ijkl} is the random residual term, ~N (0, σ^2_e).

A preliminary analysis of co-variance structure between repeated measurements using the AIC coefficient (Akaike Information Criterion, Akaike, 1974) as a model fit criteria, indicated the need to consider different co-variance structures for modeling the cow effect. For the duration and intensity of the match was used a variance component structure, Unstructured covariance matrix was used for the relative frequency of non agonistic and exhibitions, and a Huynh-Feldt covariance structure for the relative frequency of physical fights.

Differences between least squares means of the three NM levels were estimated using the Bonferroni adjusting method.

iii) Outcome of the fights

This analysis was carried out on a data set containing 188 matches, i.e. the previous 120 battle battles and another 68 matches obtained from the final tournament in Aosta. A preliminary analysis indicated no significant effects of either tournament or category on the outcome of fights, and therefore the data set was increased in order to analyse an almost equal number of fighting resulting from the three preliminary (n=93) and the final (n=95) tournaments. It is important to notice that the previous study was focussed on the differences among different levels of matches, and an equivalent number of matches among preliminary tournaments (n=3) and the final tournament (n=1) was not available. However, both datasets were consistent with the respective performed analysis.

A logistic regression analysis (SAS Institute, 2004) was then carried out with a following multiple regression model:

$$y_{ij} \sim \mathrm{Bernoulli}(p_{ij})$$
$$\mathrm{Logit}(p_{ij}) = L + OR_i + b_1{*}W_{j\,j'} + b_2{*}A_{j\text{-}j'} + b_3{*}EMVm_{j\,j'} + b_4{*}EMVf_{jij'}$$

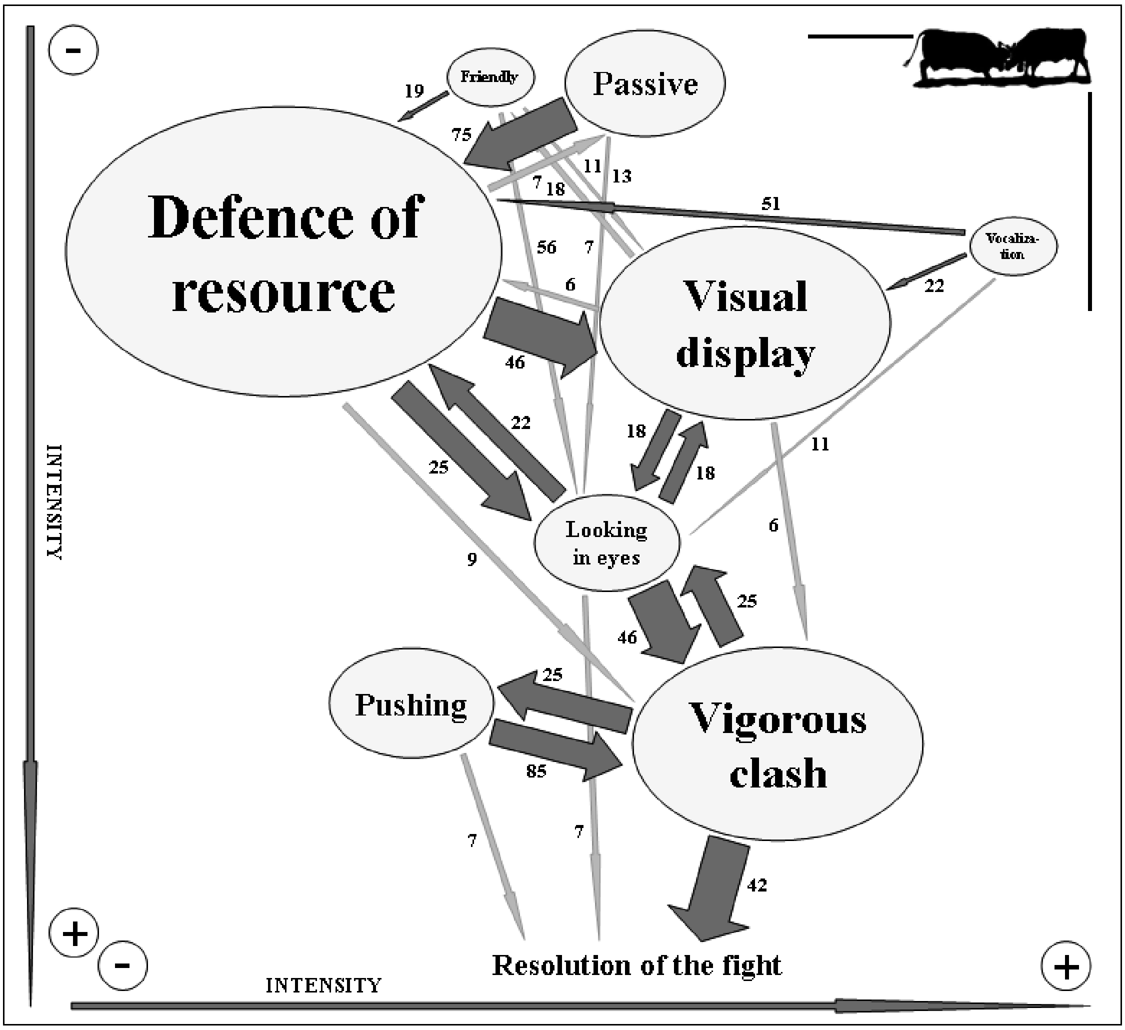
where y_{ij} is the outcome of the fight considered as binary (0 for loss and 1 for win), μ is the mean (intercept), OR is the fixed effect of the i-th order of arrival on the site of a match by the j-th cow (i=1 when arrived first and i=2 when arrived second), b_1, b_2, b_3 and b_4 are the regression coefficients of $W_{j\text{-}j'}$, $A_{j\text{-}j'}$, $EBVm_{j\text{-}j'}$, and $EBVf_{j\text{-}j'}$, respectively, that represent the individual covariates of the difference in weight (kg), age (years), estimated breeding values for muscularity trait (points) and fighting ability (points) between the target j-th individual and its opponent (j'-th); e_{ij} is the random residual term, ~N (0, σ^2_e). It should be noted that EBVs for muscularity were obtained from linear type score of primiparous cows, whereas EBVs for fighting ability were computed from fighting data by Sartori and Mantovani (2010).

RESULTS

i) Diagram of behaviours

An exhaustive summary of cow agonistic performance is provided by the transitional diagram of behavioural patterns performed in the course of a battle as reported in Figure 2. Each elliptical area is describing a behaviour and is proportional to the average duration of behaviour, while the size of arrows is proportional to the probability of moving from one behaviour to another. In general, in the course of a conflict cows spend the greatest amount of time in defending the resource (34-51% of the total time, Figure 2 and Table 2), i.e., moving the muzzle in the earth or scraping, exhibiting in front of the opponent using visual cues (14-22%; Table 2), engaging vigorous clashes (19-26%; Table 2) aiming to overwhelm the rival. Following the arrows in Figure 2, a typical pattern of escalated conflict can be found out: cows can show an initial passive behaviour or, more commonly, they can start to exhibit their right over resource as soon as they arrive. The exhibition generally (i.e., 46% of the time,

Figure 2. Transition diagram of behaviours$^{(\dagger)}$ obtained from ethograms of winners in 120 selected matches with each of the eight behavioural patterns ordered on the basis of a gradient of intensity.



$^{(\dagger)}$Areas of ellipses are proportional to the duration of each behaviour. The probability (in percentage) of a transition from a behaviour to another one is indicated by arrows; transitions with probability less than 6% are not shown, and lighter or heavier arrows refer respectively to transitions occurring less than 15% or more than 15%.

Figure 2) evolved in a visual assessment with threatening postures, culminating in a quick look into opponent’s eyes (18% of times; Figure 2) that is the signal for the battle; in half the cases, the glance led to a physical contrast, characterized by a vigorous clash that can be alternated with moments of head to head or body pushing (25%; Figure 2), that led in most cases to the resolution of the match (42%, Figure 2).

ii) Shape of the conflicts

Among the different factors considered in the mixed model ANOVA (Table 3), the tournament, category and their interaction showed in general little influence on the five variables related to fighting. Specifically, the tournament affected significantly (*P*<0.05; Table 3) only the duration of the match, while the weight category did not show any significant effect (*P*>0.05) on any of the measured variables. On the other hand, only transient significant effects (*P*<0.05; Table 3) were observed for interaction between tournament and category as regard the incidences of exhibition and physical fight on the total time of the mach. Both weight and age differences between contenders (i.e., RHP factor and winner–loser effect, respectively) showed a greater magnitude than previous variable (greater F value; Table 3) on some of the analyzed variables, and particularly on the incidence of exhibition and on the intensity of the match, that were affected (*P*<0.05) by the weight and age differences respectively (Table 3). Positive value of the estimated coefficients on weight (0.07) and age (0.01) indicated that an increasing difference in weight or age between contenders resulted in a significant increase in intensity of the score (+7% per +1 kg of weight difference) and of exhibition (+0.1% per +1 year of age difference), respectively. However, among all factor analyzed, the stage of the tournament in which cows fights, i.e., the progressive number of matches disputed, was the primary source of variation in affecting all fight variables, except for the duration of the matches, that was not significantly influenced by the number of encounters (Table 3).

Table 3. Results of the mixed model analyses (F values and significance) aimed to identify the differences in the shape of conflicts among cows.

Variable	Tournament (T)	Category (C)	T*C	Match disputed	Weight Diff. (Kg)	Age Diff. (years)
Duration of match (sec)	3.82*	0.52	1.03	1.13	0.51	2.14
Intensity of match (score)	1.53	0.74	1.26	7.94***	3.34	3.93*
Non agonistic behav./total (%)	1.84	1.48	0.62	8.88***	0.08	5.84*
Exhibition/total (%)	0.45	0.46	2.58*	3.78*	3.89*	0.98
Physical fight/total (%)	0.64	2.10	2.45*	6.86***	2.95	3.14

*=$P \leq 0.05$; **=$P \leq 0.01$; ***=$P \leq 0.001$; where not present F is not significant, i.e. $P > 0.05$.

Table 4. Least squares (LS) means with standard error in brackets and results of comparison between LS means of different number of encounters (i.e., match disputed) carried out with Bonferroni adjustment method.

Variable	Match disputed		
	1st	3rd	5th
Duration of match (sec.)	210.1 (28.6)	269.5 (27.9)	242.1 (40.5)
Intensity of match (score)	3.34 (0.11)^A	3.79 (0.11)^B	4.04 (0.15)^B
Non agonistic behavior/total (%)	0.11 (0.03)^a	0.12 (0.02)^a	0.04 (0.01)^b
Exhibition/total (%)	0.74 (0.04)^a	0.57 (0.04)^b	0.59 (0.05)^b
Physical fight/total (%)	0.15 (0.03)^A	0.31 (0.04)^B	0.37 (0.06)^B

Superscript A and B indicate differences at $P \leq 0.01$ within row; a and b indicate differences at $P \leq 0.05$ within row.

Contrasts aimed to investigate the influence of successive fights on the agonistic approaches among two conspecifics (Table 4) indicated that moving from the first to following encounters (3rd and 5th) resulted in a greater intensity (3.34 vs. 3.79 and 4.04, respectively; Table 4). This increased intensity was linked to a reduction of incidence in non-agonistic (significant only at the 5th match; Table 4) and exhibition behavior (0.74 vs. 0.57 vs. 0.59 at 1st, 3rd, and 5th match, respectively; Table 4) and to an increase in the incidence of pushing and vigorous clash (i.e., physical behavior) of about 19% (i.e. 0.15 vs. 0.31 vs. 0.37 at 1st, 3rd, and 5th match, respectively; Table 4).

iii) Outcome of the fights

As in the previous analysis, differences in weight and age were important in determining the outcome of a match (P<0.05; Table 5) that reached the value of 1.01 for weight difference and 0.81 for the age difference (Table 5). In spite of the different confidence interval, much greater for the age than for the weight difference between contenders (Table 5), this indicates that heavier cows have a greater odd (about 0.04% more than lighter) for any kg of difference in weight to succeed in the match or, on the other hand, the cow resulting 1 year younger than the contender has an odd of 0.45 to lose the match. On the contrary, the order of arrival of cows at the arena didn't affect at all the outcome of the match (Table 5). The greatest influence on the result of the battle was the estimated breeding values (EBVs) for fighting ability, revealing a strong significant role (*P*<0.001; Table 5) in influencing the probability of a win or a loss in the fight. Particularly, the odds ratio obtained for difference in EBVs for

Table 5. Results of logistic regression analysis carried out on the outcome of the fight considered as binary (0 for defeat and 1 for win).

Item	Wald χ^2 and significance	Odds Ratio	95% Wald confidence limits of odds ratio	
			Upper	Lower
Intercept	3.25	-	-	-
Order of arrival on fight	1.25	1.51	0.73	3.10
Difference in weight (kg)	4.37*	1.01	1.00	1.02
Difference in age (years)	3.80*	0.81	0.66	0.99
Difference in EBV for muscularity (points)	2.10	1.01	0.99	1.02
Difference in EBV for fighting ability (points)	35.43***	1.08	1.01	1.11

*=$P \leq 0.05$; **=$P \leq 0.01$; ***=$P \leq 0.001$; where not present F is not significant, i.e. $P > 0.05$.

fighting ability among contenders indicated a grater odd (i.e., 4% more than loser) to win a match when differences in EBVs increase by 1. Such result reflects about 14 points of phenotype considering a heritability of 0.07 for the trait (Sartori and Mantovani, 2010). On the other hand, no effects were attributable to the EBVs difference between contenders as regard the muscularity (Table 5)

DISCUSSION

Agonistic interactions among conspecifics are increasing in popularity in animal behaviour, and the initial theory describing ideal animal conflicts (theory of games; Maynard Smith and Price, 1973) has recently been expanded and developed with new inputs and suggestions aimed to explain how disputes really happen in nature (see Arnott and Elwood, 2009 and Hsu *et al.*, 2006 for reviews). In such a framework, agonistic interactions occurring among cows are able to provide a good example of the dynamics of animal conflict, providing a concrete scenario for confirming important theoretical concerns. In our study, the transitional diagram of behavioural patterns exhibited by cows in the course of the struggles seems a clear example of common agonistic dynamics occurring among conspecifics. Agreeing with theory, when the cost of the fight is elevated (i.e., the physical effort that individuals have to provide into the battle), competitors are used to escalate (Parker, 1974; Clutton-Brock and Albon, 1979), giving each other indications about their own fighting ability through honest signals with increasing costs and thus more reliable information (Zahavi, 1975; Enquist and Leimar, 1983). Our diagram clearly demonstrate that behaviour in cattle fighting reflect these escalated pattern and it is similar to diagrams for escalated conflicts built in other taxa (i.e., roe deer, *Capreolus capreolus*, Hoem *et al.*, 2007; whip spider *Phrynus marginemaculatus,* Fowler-Finn and Hebets, 2006). In addition, our observation on cows agonistic behaviours as threatening displays or pushes have been already described both in related species such as bison (*Bison bison*, Roden *et al.*, 2005) or buffalo (*Syncerus caffer,* Sinclair, 1977) and in other cattle breeds (i.e., Holstein Friesian, Collis, 1976, Gibbons *et al.*, 2009; Scottish Highland cattle, Reinhardt *et al.*, 1986). Moreover, also in other traditional tournaments with Heréns breed such patterns have previously been mentioned (Bouissou and Boissy, 1995). The transitional diagram of behavioural patterns here presented could also be considered in agreement with the “cumulative assessment game” theory of Payne (1998), where competitors may decide to escalate after assessing both reciprocal asymmetries and own RHP.

Following Maynard Smith and Parker (1976), disputes of fighting cows, aimed to establish relationships of social dominance, reflect asymmetries in RHP occurring among contenders. Our results confirm such theory, indicating that when asymmetry between contender increase (i.e., weight differences), the conflict results in greater exhibition of strength and, as a consequence, in greater intensity of the match. This could be considered to agree with the “partial mutual assessment” theorized by Prenter *et al.* (2006), in which both absolute weights and the difference in size between contenders play a role. However, results obtained analysing the “Batailles of Reines” may suggest something more. Indeed, studies carried out in last decade on fighting have shed light on the great importance of the winner-loser effects (Hsu and Wolf, 1999; 2001), explaining that also factors other than RHP, such as prior experiences, physiological variations, influence the shape and outcome of a fight. In light of this, the significant age effect observed in this study on the outcome of cow fights confers to the previous experiences collected over years an important role for the resolution of the struggles. However, an important outcome of this study could be related to the evidence that struggles change over time, after experienced repeated agonistic interactions (i.e., previous encounters). As time went by, the level of intensity increased, as the number of physical interactions, whereas the amount of exhibitions and non agonistic interactions reduced. Results of this study clearly indicate this pattern. Temporal variations of fights have been already observed in fishes, when new colonies are established (Oliveira and Almada, 1998), and when individuals ascend in ranks during hierarchic assessments (Wong and Balshine, 2010), but literature is still quite scarce on such a topic. Changes in physiological conditions have been described as a primary factor unrelated to RHP but affecting shape and sorts of conflicts (Rutte et al., 2006), as demonstrated in laboratory mice (i.e., *Peromiscus californicus*, Bondar *et al.*, 2009; *Mus musculus*, Oyegbile *et al.*, 2005), where variations in levels of androgens influence new fighting performances. Stress hormones (Creel, 2001) and testosterone (Wingfield, 2005) play a key and antagonistic role as enhancers and reducers of fighting effort, respectively. Moreover, it has been demonstrated that levels of testosterone may increase and subsequently slowly decrease in the minutes just after an agonistic interaction, reaching the pick around 45 minutesafter the contest, and thus enhancing the aggressiveness in individuals (Oyegbile *et al.*, 2005), that may be exhibited in further conflicts. The rise of intensity in the course of the “Batailles of Reines” reflects such dynamics, although no physiological data could have been obtained and analyzed in our study. Therefore, we can only speculate that the greater level of aggressiveness and thus of agonistic intensity in the course of the tournament could be related to physiological changes as those previously described.

Among the results obtained in this study, the value of the resource did not seem to be important for the outcome of the fight, as shown by the logistic regression analysis. Indeed, the order of arrival at the patch (first or second) did not affect the probability to win. Such observation does not agree with the bourgeois theory of Davies (1978), for which the first arrived is expected to perceive the resource as “own” and thus to invest more in the conflict, enhancing the probability to win. That could be due to the latency at the piece of earth before opponent’s arrival, maybe not sufficient for enhancing a perception of ownership on the resource. The importance of the genetic value (EBVs) for fighting abiliy observed in this study (i.e., EBVs assessed in Sartori and Mantovani (2010)) agrees with previous reports that hypothesized a role of the genetic value in conflicts (Kondo and Hurnik, 1990; Murphy and Duarte, 1990). The role of genetics in behavioural traits has been studied over decades (Boake, 1994) and has received new vigour from recent theories of interacting phenotypes and indirect genetic effects on social traits (Moore *et al.*, 1997; Bijma *et al.*, 2007b). The genetic estimation of fighting ability in cows (Sartori and Mantovani, 2010) and bulls (Silva *et al.*, 2006) well proved the important role of genetics in such traits. As a matter of fact, domestication and selection for breeding purposes has modelled some relevant behavioural traits in livestock for centuries (Grandin, 1997; Mignon-Grasteau *et al.*, 2005), playing a role of major importance for animal welfare and productive purposes.

CONCLUSIONS

Fights among cows occurring in the course of traditional tournaments are of great interest in investigations of animal conflicts. A typical pattern of escalated conflict based on the defence of the resource, exhibition and fighting has been demonstrated. Analyses carried out in this study indicated that physical factors related to RHP such as weight, and aspects related to previous experiences as age, play an important role in affecting the shape and the outcome of a conflict. Furthermore, the recent experience of repeated encounters can reveal as the most important factor in influencing the intensity, and the remarkable impact of genetic components can shed a new light on agonistic studies and concerns.

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Chapter 2

Running head: Genetics of fighting cows in Valdostana breed

GENETICS OF FIGHTING ABILITY IN CATTLE USING DATA FROM THE TRADITIONAL BATTLE CONTEST OF VALDOSTANA BREED

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"BEHAVIOR is (...) the most complex phenotype that can be studied because behavior reflects the functioning of the whole organism and because it is dynamic and changes in response to the environment."

(Plomin, 1990)

ABSTRACT - The tendency to fight is a well known attitude in Valdostana cattle, and non-cruel traditional contests are organized yearly by farmers to identify the most dominant cow. Cow battles consist of eliminating matches that have important economic implications both for tourism and farmers. The aims of this study were i) to validate a score system to express fighting ability, and ii) to carry out a genetic analysis for this trait using different datasets and models. A dataset including 16,509 fighting records from 5,981 cows relevant to contests over six years was retained after editing (dataset1). Data on placements were used to compute a placement score (PS) accounting for wins, tournament size and difficulty, and differentiating the 20 preliminary battles each year from the final match. A second dataset was created using only the individual best yearly PS, i.e. deleting repeats with a lower PS for the same animal within each year (dataset2; n=10,367 records, corresponding to a single datum per year per cow). Compared to the cows' placement or position (P), the PS was less skewed (-1.45 for P and 1.25 for PS, respectively) and exhibited better coefficients for the probability of a normal distribution. Animal model REML method analysis (accounting for 13,456 animals in the pedigree) was carried out considering different combinations of fixed and random non-genetic factors other than the random animal and permanent environmental effects. Results indicate that random factors other than the additive genetic and permanent environment do not improve the model fit and therefore it is not useful to take them into account. Heritability estimates obtained with the model showing the best fitting, were 0.078 (dataset1) and 0.098 (dataset2). Results of this study indicate that selection for fighting ability in Valdostana cattle using battle data of performances could be possible.

Key words: genetics, fighting ability, cattle, battle contest, Valdostana breed

INTRODUCTION

In all social species the access to resources is regulated through dominance relationships involving repeated dyad agonistic interactions generating a within-group hierarchy of social dominance (Drews, 1993). Rigid relations are typical of confined ungulates living in groups such as bison, zebus and cattle (Reinhardt et al., 1986). In cattle, herds firm hierarchies are always established and the regrouping at pasture or the re-mixing of unfamiliar animals produce aggressions aimed at defining a new social order (Phillips, 1993; Bøe and Færevik, 2003). Dominance shows a heritable component, but few studies on cattle have shed any light on it (Dickson et al., 1970; Wieckert, 1971). Fighting ability has been investigated in breeds used for bull fighting and selected for aggressiveness (Gonzalez Caicedo et al., 1994; Silva et al., 2006). Genetic analyses have been also carried out for Hérens and Valdostana cattle using data from the traditional cow battle contests (Plusquellec and Bouissou, 2001; Mantovani et al., 2007). These competitions between cows represent both an attraction for tourists and a source of income for farmers, due to the increased economic value of the most competitive cows and their offspring. Although selection for fighting ability has not yet been carried out formally, farmers pay a great deal of attention to this trait in addition to the selection for the dual purpose attitude. As part of a project aimed at identifying the possible implementation of genetic selection for fighting ability in the Valdostana breed (Mantovani et al., 2007) this study aims at: i) validating a score system as a suitable dependent variable to analyze fighting ability, ii) investigating different combinations of fixed and random effects in different datasets to identify the best-fitting model. As an outcome, variance components and rank correlations among estimated breeding values are analyzed and discussed.

MATERIALS AND METHODS

Data used in the study were obtained following the guidelines given by the association of farmers responsible for the battle organization. These guidelines are formulated in respect to Italian legislation on animal care.

Description of the subject

The “Batailles de Reines” are annual traditional contests that have taken place since 1958 in the Valle d’Aosta region, i.e. north-west Italian Alps, in which cows dispute eliminatory bloodless matches aimed at identifying the most competitive animal (Mantovani et al., 2007). Contests revive the natural attitude to fight that cows exhibit at the beginning of the summer grazing season, when unfamiliar cows meet after regrouping. The fights are carried out in grass arenas, where pairs of cows are left to fight under the supervision of their owners and a judge. Participants are divided into three weight categories that battle at the same time but without interactions between categories. The escalated fight (Parker, 1974; Clutton-Brock and Albon, 1979) can end quickly if a cow immediately recognizes the superiority of its rival, but it may last even more than an hour, with cows pushing each other until the loser gives away. When a cow recognizes the hierarchical supremacy of the adversary, it is eliminated from the competition, whereas the winner advances in the tournament. Yearly tournaments consist of 20 preliminary battles starting in the last Sunday of March and a final match that takes place at the end of the summer pasture season (i.e., about at the middle of October). The final match is held every year in a special arena in Aosta, and is disputed for each of three weight categories by all animals classified in the eliminatory tournaments (winner and up to the fourth placements) plus the winner of the previous year. The winners of each category gain the title of “queen” of the year. Both the eliminatory and final battle boards are established within each category and across tournaments by drawing animal numbers, i.e., without seeds. Only cows belonging to the autochthonous Aosta Chestnut and Aosta Pie Black breeds from farms located within the regional territory are allowed to compete in the tournaments. These breeds have a strict genetic relationship (Del Bo et al., 2001) and they are considered two strains of the same breed managed within the same herd book. To fight, cows need both an ongoing or documented milk production before fighting and have to be diagnosed pregnant. Lastly, cows not classified for the final match are allowed to compete in further preliminary tournaments within the same year. It’s important to note that the “Batailles de Reines” tournament does not imply the same ethical problems that can arise from traditional dog, cock or bull fights as it is a bloodless, non-cruel competition.

Data collection and editing

Raw data on the results of fights carried out during six successive years (2001 to 2006) of the traditional “Batailles de Reines” contest were collected from the Valle d’Aosta farmer association (AREV), responsible for the organization of the battles and for the collection of fight data. The original data consisted of the results of 19,665 fighting matches performed by 7,379 cows in three weight categories, and accounted for both preliminary and final tournaments held in each year. The annual datasets were organized in pairs for participants reporting the winner and the loser of each match. These original data were edited and re-arranged to report, for each cow, the corresponding year-battle for each weight category, the individual weight at fighting, and the final position reached within the battle-board. Individual cows’ records were completed with information about the herds, the age at the battle (in years) and the genealogic information. Annual datasets were joined and data discarded if they were incomplete or if they belonged to a herd-year class with only one cow in the competition. After editing, 16,509 fighting results belonging to 5,891 cows were retained for further analysis (dataset1). This dataset could contain several fight results for the same cow

Table 1. Descriptive statistics on data of “Batailles de Reines” retained in both datasets produced after data editing and relative to 369 levels (123 year-battles x 3 weight categories) and 5,891 cows in all datasets

Item	Dataset1	Dataset2
Records, No.	16,509	10,367
Herd-year classes, No.	2,337	2,182
Participants within year-battle*category, No.	44.7±22.9	28.1±14.1
Participants within herd-year, No.	7.1±7.0	4.8±3.7
Fighting/cow, No.	2.8±2.4	1.8±1.0
Age of participant, yr	6.1±1.7	6.0±1.7
Weight of participant, kg	548±61	544±60
- weight category 1 (Heavy), kg	633±43	629±42
- weight category 2 (Medium), kg	545±20	543±19
- weight category 3 (Light), kg	495±22	492±22

within a particular year. Another dataset (dataset2) was created from the previous one, keeping only the best yearly performances of each cow within a year (10,367 fighting records) and discarding other performances that had led to poorer results. In spite of the non-random choice of records, dataset2 aimed to reflect the tendency of breeders to bring animals to more than one tournament when they are not satisfied by cow's placement (mainly due to the absence of seeds). Descriptive statistics concerning the two datasets are reported in Table 1. Because there were no changes in the actual number of individual cows included in both datasets, a single relationship matrix was set up containing all available pedigree information. As a result, a total of 13,456 animals were retained in the pedigree file for subsequent analysis. Taking into account the maximum number of generations traced for each individual, an average of 2.3 mean numbers of generations per cow were considered. Moreover, individuals in pedigree referred to 858 sires drawn from artificial and natural insemination programs, for an average half-sib family size of 6.1 daughters per sire.

Scoring the position in each battle

Because the rank in the battle board has a skewed distribution (Mantovani et al., 2007), a scoring of the position was developed to obtain an almost normal distribution to be used in the subsequent analysis. Thus, a dominance index based on the results of dyadic interactions of participants in the tournaments was computed. However, unlike the previous placement score (PS; Mantovani et al., 2007), the present one was formulated by combining the suggestions for scoring a place value, as reported by Langlois (1984) and modified from Dorofejew and Dorofejewa (1976), with a relative place number, as reported by Bruns (1981) and attributed to H. Shertler (unpublished data). Both these methods, previously analyzed by Mantovani et al. (2007), were derived from a scoring system of placement for horses in jumping and dressage competitions. In the present study, the PS accounted both for the number of wins obtained by each cow in a specific tournament, correcting for the number of participants in the competition (from 16 to 153) and giving a different value to the final match as compared to the preliminary battles. In synthesis, the PS could be summarized by the following formula:

$$PS_{ijkl}=20+ty_i+2w_j+d_k \qquad (1)$$

where PS_{ijkl} represents the score of cow l in a given tournament, depending on the type of tournament ty_i (with ty=0 for i=eliminatory tournaments and ty=7 for i=final tournament in Aosta), on the number of wins w_j obtained by each animal in the given tournament-category (with j=0,…, 8) and on a difficulty coefficient of tournament d_k related to the number of participants in the tournament-category linked to the battle-board size (5 classes with k=-2: >128 participants, -1: 65-128, 0: 33-64, 1: 17-32 and 2: <17 participants, respectively). An arbitrary constant value of 20 was added to the final PS to avoid negative values. Table 2 shows all possible values of PS in preliminary tournaments applying equation (1).

Table 2. Possible values of Placement Score with the number of wins achieved by individuals in parenthesis. All the scores of the final battle in Aosta get 7 points of increment from the depicted values

No. of participants[a]	Position achieved by a participant in battle board								
	1st	2nd	3rd-4th	5th-8th	9th-16th	17th-32th	33th-64th	65th-128th	>129th
0-16	30 (4)	28 (3)	26 (2)	24 (1)	22 (0)				
17-32	31 (5)	29 (4)	27 (3)	25 (2)	23 (1)	21 (0)			
33-64	32 (6)	30 (5)	28 (4)	26 (3)	24 (2)	22 (1)	20 (0)		
65-128	33 (7)	31 (6)	29 (5)	27 (4)	25 (3)	23 (2)	21 (1)	19 (0)	
>128	34 (8)	32 (7)	30 (6)	28 (5)	26 (4)	24 (3)	22 (2)	20 (1)	18 (0)

[a] No. of participants: number of contestants in a given battle board within weight category.

Models and analyses

The UNIVARIATE procedure of SAS (SAS Institute, 2004) was applied on dataset1 for a preliminary comparison of the distribution of PS and the simple individual placement (POS). A subsequent analysis of variance on non-genetic effects treated as fixed effect was performed on each dataset using the GLM procedure of SAS (SAS Institute, 2004), aimed at identifying the magnitude of each possible source of variation. With the exception of the breed variety (Chestnut or Black Pied), all non genetic effects taken into account in the ANOVA produced a significant effect on the PS ($P<0.001$; data not presented), with a final R^2 of 0.45 and 0.50, in dataset1 and dataset2, respectively. The non genetic factors included in the genetic analysis were: the effect of the year-battle by weight category (YB*C, 123 different year-battles by 3 categories for a total of 369 levels), the herd-year effect (HY, with 2,337 different levels in dataset1 and 2,182 in dataset2), the effect of the class of age of participants (AC, 7 classes: ≤3, 4, 5, 6, 7, 8 and ≥9 years of age at fighting), and the individual weight as covariate within each weight category (b*W, 3 levels). Preliminary ANOVA indicated that all these factors could be retained in the final animal model because of no variance overlapping among them could be detected.

The subsequent analysis aimed at estimating variance components was carried out with a single trait animal model (expectation-maximization-REML method) using the appropriate program from the BLUPF90 family (Misztal, 2008). In the genetic analysis the comparison of dataset1 and dataset2 was also carried out considering different combinations of fixed and random non-genetic factors other than the random animal and permanent environmental effects. Model 1 considered the YB*C and the HY as fixed effects, while models 2 to 4 considered different combinations of YB*C or/and HY as random effects. Therefore, the most complete matrix notation of the models can be expressed as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{W_1q_1} + \mathbf{W_2q_2} + \mathbf{W_3p} + \mathbf{Zu} + \mathbf{e}$$

where $\mathbf{y}$ is an N×1 vector of observations, $\boldsymbol{\beta}$ is the vector of systematic fixed effects of order p, $\mathbf{q_1}$ is the vector of year-battle*category when considered as random effect (model 2 and 4), $\mathbf{q_2}$ is the vector of herd-year of order z when considered as random effect (model 3 and 4), $\mathbf{p}$ is the vector of permanent environmental effects of order q, $\mathbf{u}$ is the vector of animal effects with order m, and $\mathbf{e}$ is the vector of residual effects. Furthermore, $\mathbf{X}$, $\mathbf{W_1}$, $\mathbf{W_2}$, $\mathbf{W_3}$, and $\mathbf{Z}$ are the corresponding incidence matrices with the appropriate dimensions.

The assumptions about the structure of (co)variance were, in the model with the greater number of random factors, as follows:

$$\mathrm{Var}\begin{bmatrix}\mathbf{a}\\ \mathbf{p}\\ \mathbf{y}\\ \mathbf{h}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma_a^2 & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}\sigma_p^2 & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_y^2 & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_h^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{I}\sigma_e^2\end{bmatrix}$$

where σ_a^2 is the additive genetic variance, σ_p^2 is the permanent environmental variance, σ_y^2 is the YB*C variance (in model 2 and 4), σ_h^2 is the HY variance (in model 3 and 4), σ_e^2 is the random residual variance, $\mathbf{A}$ is the numerator relationship matrix, and $\mathbf{I}$ is the identity matrix.

For all datasets and models investigated heritability (h^2) and repeatability (r) of fighting ability were estimated as follows:

Heritability:

$$h^2 = \frac{\sigma_a^2}{\sigma_t^2}$$

Repeatability:

$$r = \frac{\sigma_a^2 + \sigma_p^2}{\sigma_t^2}$$

Where σ_t^2 is the total phenotypic variance, given by the sum of all estimated variance components.

Due to software limitations, the standard errors (SE) for heritability estimates were approximated using the following formula (Falconer, 1989):

$$SE_{\hat{h}^2} = 4\sqrt{\frac{2(1-t)^2[1+(k-1)t]^2}{k(k-1)(s-1)}}$$

Where t is the intraclass correlation approximated by ($h^2/4$) for paternal half-sib estimates, k is the average number of offspring per sire, and s is the number of sires obtained from the pedigree file.

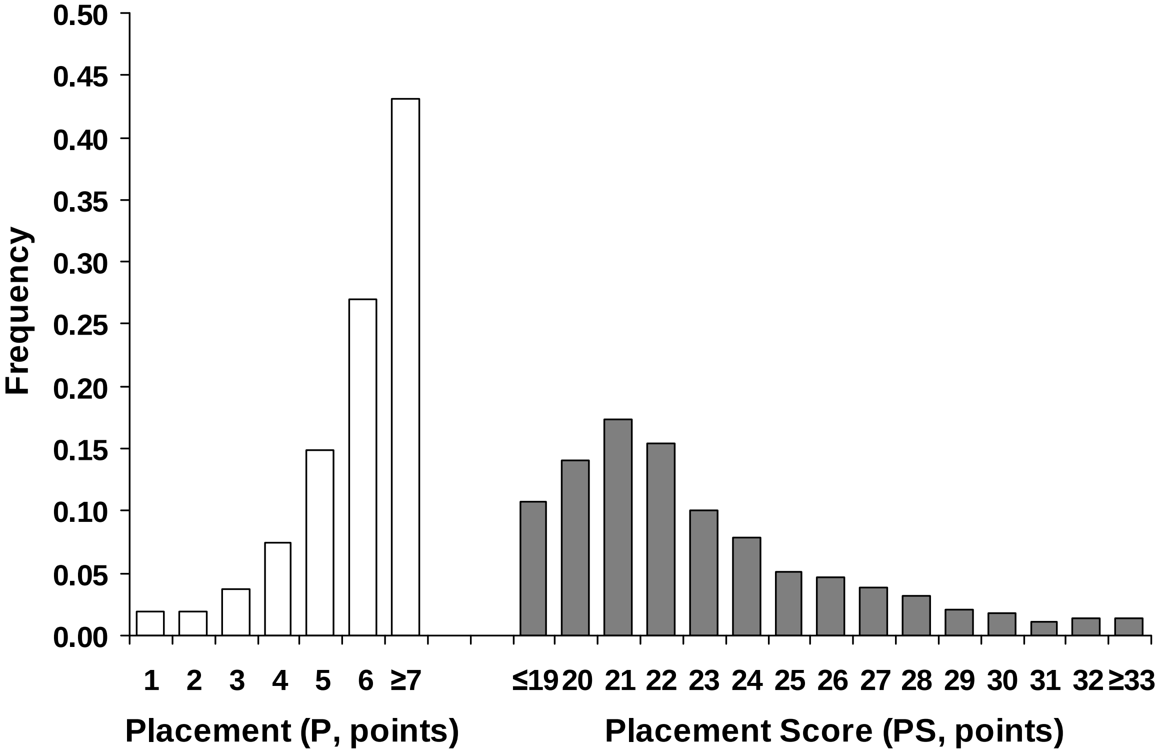
A comparison between the values of Akaike Information Criterion (AIC; Akaike, 1974) was used to evaluate how well the models fitted in all scenarios (models and datasets) as in examples provided by Burnham and Anderson (2002).

Rank correlations between estimated breeding values (EBV) in both datasets while only using the model that showed the best fitting (model 1) were also obtained separately for animals with fighting records (n=5,891) and for their sires (n=858) using the CORR procedure of SAS (SAS Institute, 2004).

RESULTS

The distribution of the placement (P) and placement score (PS) obtained applying the described formula to the complete dataset containing 16,509 records retained for analysis are presented in Figure 1. Placement score was closer to the normal distribution than placement (P), as the lower Kolmogorov-Smirnov and Anderson-Darling values indicated (data not shown in tables). Both distributions proved to be skewed, but PS shows a lower absolute value of the skewness coefficient as compared to P (1.25 vs. -1.45 for PS and P, respectively), still indicating that PS was closer to a normal distribution than P, as shown in Figure 1.

Figure 1. Frequency distribution of data retained for the study and expressed as Placements (i.e. final position in the battle board indicated with white bars on the left) or as Placement Score (dark grey bars on the right calculated from equation (1) in Materials and Methods) used to express fighting ability of each cow that fought in a year-battle*category of weight



Sartori & Mantovani (Genetics of fighting cows in Valdostana breed) – Figure 1

Indeed, the distribution of P is almost totally asymmetric (Figure 1). This is due to the fact that each subsequent position after the winner and the second classified animals (which have the same frequency), presents almost double the frequency compared to the next position, due to the structure of the battle board used for fighting.

Table 3 reports REML estimates obtained with different models and datasets. The dataset containing only one annual individual performance (dataset2) and the model that accounts for both YB*C and HY as fixed factors (model 1) gave the best fit to the data, as revealed by the lower value of AIC (Table 3). Heritability estimates range from 0.068 (model 2, dataset1) to 0.148 (model 3, dataset2). In all cases, the dataset including only the best yearly performance of a cow (dataset2) produced greater heritability estimates. In the analysis that produced the best fit (i.e., model 1, dataset 2), heritability was 0.098. The standard error of heritability estimates was on average 0.043, with a reduced range of variation among datasets and models (from 0.042 to 0.044; data not shown). The repeatability was on average 0.24, and ranged from 0.21 in the model including both YB*C and HY as random factors, to 0.28 when only HY was considered random. The ratio of the permanent component to the total variance was on average (dataset 1 and 2) 0.138, revealing a slightly greater magnitude of the permanent component than the additive genetic component. The rank correlation among breeding values estimated in the two datasets for model 1 was 0.915. This correlation, when limited to the 858 sires, reached a similar value of 0.924.

Table 3. Variance components, Akaike Information Criterion (AIC) estimates, heritability (h^2), and repeatability estimates (r) obtained with different models and datasets used

Item	Variance components[1]					AIC	h^2	r
	σ^2_y	σ^2_h	σ^2_a	σ^2_p	σ^2_e			
Model 1[2]								
- Dataset1[3]	-	-	0.591	1.295	5.731	77,615	0.078	0.248
- Dataset2[4]	-	-	0.752	1.216	5.735	48,380	0.098	0.255
Model 2[5]								
- Dataset1	1.236	-	0.609	1.387	5.667	78,818	0.068	0.224
- Dataset2	1.917	-	0.796	1.343	5.605	49,774	0.080	0.221
Model 3[6]								
- Dataset1	-	0.156	0.869	1.014	5.751	85,570	0.112	0.242
- Dataset2	-	0.228	1.190	1.029	5.614	56,431	0.148	0.275
Model 4[7]								
- Dataset1	1.489	0.184	0.857	1.085	5.695	86,817	0.092	0.209
- Dataset2	1.995	0.244	1.210	1.113	5.528	57,806	0.120	0.230

[1] Variance components: *σ^2_y =year-battle*category, σ^2_h =herd-year, σ^2_a =additive genetic, σ^2_p = permanent environmental, and σ^2_e =random residual.

[2] Model 1: YB*C and HY both treated as fixed effects;

[3] Data set with all performances for each cows, 16,509 records.

[4] Data set with the only year best performance for each cow, 10,367 records.

[5] Model 2: YB*C treated as random and HY treated as fixed effect.

[6] Model 3: YB*C treated as fixed and HY treated as random effect.

[7] Model 4: YB*C and HY both treated as random effects.

DISCUSSION

In comparison to our study, other research on social dominance has been based on the registration of the results of dyad encounters of members within a social group, used for assigning competitive values to individuals (De Vries, 1998; Langbein and Puppe, 2004; Val-Laillet et al., 2008). Agonistic interactions occurring in a group of cows are generally recorded within a herd during a given period of time and plotted to obtain an almost linear hierarchy (e.g. Beilharz and Zeeb, 1982; Reinhardt et al., 1986) and a consequent "aggressive order" (McGlone, 1986). Dominance relationships among individuals can also be investigated by forcing animals to engage in dyadic agonistic interactions in standardized environments. A "competitive order" (Syme, 1974) is thus obtained. The "Batailles de Reines" represents an ideal scenario for assessing dominance relationships through a "competitive order" thanks to its peculiar structure of dyadic agonistic interactions between a huge numbers of animals under the same conditions, although interactions between all the members of a group cannot be investigated using data from the battles, because after a defeat an animal is obliged to withdraw from the contest. Starting from this point and considering the importance of a symmetric distribution of fighting results, this study has attempted to identify a suitable scoring system for the fights. With this in mind, the Placement Score was designed to take into account the number of wins each animal achieved within a tournament (i.e. more victories, greater score) corrected according to the number of participants in the tournament (a large number of participants can give more chance of getting a win). Moreover, by assigning a different weight to preliminary tournaments as compared to the final match, a possible overlapping of scores is avoided. In addition, the difficulty coefficient allows a greater score to be assigned to matches within small tournaments, where the probability of fighting against the final winner or another well-classified cow at the same level of competition is higher due to the reduced number of opponents. In general, the PS resulted in a fairly normal distribution, mitigating possible statistical analysis problems due to the asymmetric distribution of the simple placement in the battle board.

However, further steps in modeling the dyadic data of "Batailles" could be considered in the future, particularly aiming at correcting the PS for the strength of the competitor, or using a model better suited to analyze ranks, such as the thurstonian model (Gianola and Simianer, 2006). Alternatively, the competitor effect may be accounted for in the genetic model, as has been done in studies on genetic effects in social behavior (Moore et al., 1997; Bijma et al., 2007). Possible comparisons of PS with the literature are not easy because of the absence of population studies on fighting ability based on tournaments.

In a pilot study on social dominance in cattle, Schein and Fohrman (1955) observed that age and weight are important factors associated with fighting performances and dominance. Our results confirm that both age and weight can influence the placement score and consequently the fighting ability of Valdostana cows. However, in our study, we attempted to identify also possible factors related to the specific contest of each fight (year-battle*category; YB*C), and with the behavioral background of individuals belonging to different herds, that could be mirrored by the herd-year (HY) effect. With regard to the YB*C effect, it tells us exactly which challenge an individual took part in, allowing for the adjustment of a cow's performance based on the performances of the other participants in the same battle contest. Moreover, the HY effect is aimed at reflecting the within-herd social hierarchy and fighting background that can change over the years due to variation in herd composition and that can influence the individual's perception of its own fighting ability and thus fighting performances. In the present study, the data structure and the reduced size of half sib families (6.1 daughters/sire) could have biased the genetic parameter estimates, although it is not easy to quantify the exact amount of such biases. Other possible biases could be due to the non-random choice of records in dataset2, i.e., retaining only the best yearly cow's performance. This could inflate the heritability estimates. However, the generally low heritability estimated in our study is in agreement with literature values obtained for other behavioral traits (Hohenboken, 1986; Mousseau and Roff, 1987), reflecting a strong behavioral plasticity that allows individuals to adapt to varying environments. Heritability estimates for social dominance ranged from 0.07 (Dickson et al., 1970) to 0.40 (Beilharz et al., 1966) in Holstein dairy cattle. Genetic evaluations carried out on agonistic performances of fighting bulls (i.e., Lidia cattle breed), revealed a heritability of 0.19 for bullfighting in a Colombian herd (Gonzales Caicedo et al., 1994), and around 0.30 for Spanish bullfights (Silva et al., 2006). A preliminary estimation of heritability for fighting ability was also obtained by analyzing traditional tournaments for Hérens cows in Switzerland (Plusquellec, 2001). The scoring method applied to evaluate fighting performances came from the ranking applied in horse competitions (Tavernier, 1991), and the heritability estimate was 0.045.

Behaviour typically includes several different environmental factors (i.e. learning, social interactions) as well as a genetic component. In this study the values of repeatability obtained indicates that environmental effects are predominant with respect to additive components.

This could be due to the kind of phenotype measured, which is modeled by previous experiences with other counterparts and mainly recorded at adult age.

The correlation between ranks of estimated breeding values derived from an analysis of the two different datasets shows only small changes in animal ranking, indicating substantial uniformity among datasets, in spite of possible overestimates of heritability due to the non random choice of records in dataset2. Therefore, genetic indexes derived from this study may led to possible application for selection. As a matter of fact, the knowledge of genetic components in behavioral traits could be important for developing strategies to genetically modulate behavior expressions, as has already been done for docility in Limousine cattle (Phocas et al., 2006). Future studies accounting for alternative scoring methods or different models, as eventual genetic correlation between behaviour and productive traits, could be useful for better understanding fighting ability and its role in animal welfare and management.

CONCLUSIONS

This study indicates that genetic evaluation and selection for fighting behavior are possible, although the proportion of additive genetic component of the trait is low. However, this is in agreement with the heritability estimates for other behavioral traits, especially when related to social dominance and fighting. The proposed placement score obtained from battle board seems a possible way to express phenotypic values useful for genetic evaluation and also to address selection for fighting ability.

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Chapter 3

Running Head: IGEs in social dominance

GENETIC BASES OF SOCIAL DOMINANCE: INDIRECT GENETIC EFFECTS IN COW FIGHTING ABILITY

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“Conspecifics rivals are an
environmental contingency
that can itself evolve”
(West-Eberard, 1979)

ABSTRACT - Genetic studies of social behaviour have currently received new impetus from recent models including the indirect genetic effects provided by social partners, assessed through different approaches. This study is aimed at investigating the contribution of conspecifics in the genetics of social dominance. At first, the possibility of accounting for the social partner either within phenotype or directly in the model as indirect genetic effects was considered. Subsequently, the incidence of both direct and associative variance components affecting social interactions was evaluated through hierarchical models. Social dominance was assessed in terms of fighting ability, scored during bloodless tournaments among alpine cows belonging to Aosta Chestnut and Pie Black breeds. As alternative phenotypes, an individual placement score and an equivalent score that considers competitor strength. Model comparison showed that introducing indirect genetic effects led to better estimates (i.e., lower values of AIC) than applying classical quantitative models or including the opponent in the phenotype. Heritability estimates for fighting ability ranged between 0.01 and 0.09, with generally lower values when both genetic and environmental associative variances were included. Associative genetic variance driven by indirect genetic effects was greater than the direct genetic component, whereas the introduction of associative environmental components seemed to trigger problems in variance estimation. Then again, models including indirect genetic effects were confirmed as the most suitable resource in investigating social traits.

Keywords: IGEs, social dominance, fighting ability, cows, quantitative genetics

INTRODUCTION

Genetic investigation of behavioral traits are generally complicated by the strong influence of the environment (Boake et al., 2002), and studies on social behavior (i.e., social dominance) are made more intricate because of interacting partners that are both the action and reaction of focal behavior (i.e., “interacting phenotypes”; Moore et al., 1997). A mathematical representation of this theory was given by Moore et al. (1997), who introduced the concept of “indirect genetic effects” (IGEs) as the genetic part of the individual phenotype due to the contribution of interacting social partners. An alternative but equivalent approach for modeling social behavior via quantitative genetics was recently proposed by Muir and Schinckel (2002) and by Bijma et al. (2007a), decomposing an animal’s genotype as the sum of direct and social components due to all interacting conspecifics. The advantages of such models are the possible inclusion of both direct and associative permanent environmental components and the use of a single phenotype (Mc Glothlin and Brodie III, 2009). This could be particularly interesting to evaluate the genetics of social dominance that is characterized by fights in which both contestants can equally exhibit dominant and submissive postures, which could dramatically complicate the genetic analysis if they were treated as different traits. In this perspective, summarizing social dominance into one single phenotype, reflecting the potential social skill of one individual, may permit an easier analysis. Fighting ability (or resource holding power, Parker, 1974) is a good estimator of social dominance, reflecting different abilities to acquire resources in the presence of others (i.e., to compete; Hawley, 1999). Genetic investigations of fighting ability as an indicator of social dominance have already been carried out on data of traditional battles of bulls (Gonzales-Calceido et al., 1994; Silva et al., 2006) and cows (Plusquellec, 2001; Mantovani et al., 2007; Sartori and Mantovani, 2010). All evaluations were carried out via classical quantitative models, as in the other species in which the genetics of social dominance has been investigated (i.e., Japanese Quail, Nol et al., 2006; Bank Vole, Horne and Ylonen, 1998). A new perspective in these studies could be represented by the use of IGEs, that have already been taken into account for fighting traits in Cockroach (Moore et al., 2002) and Mouse (Wilson et al., 2009). As further development of the previous work on cow fighting ability (Sartori and Mantovani, 2010), the present study is aimed at investigating additional alternatives to the genetic analysis of the trait, including IGEs. Specifically, the objectives of the study were i) to compare genetic investigation of fighting ability carried out with a traditional genetic model or with alternative models that consider the contestants strength either as a phenotype or as a IGE; ii) to investigate the magnitude of the competitor effect in determining the phenotype of the focal individual, and iii) to quantify the incidence of IGEs on social dominance.

MATERIALS AND METHODS

Subject of the study and dataset

Data available were the results of the traditional battle contest organized annually by the farmer organizations within the Aosta region (north-west of Italy) as a revival of the agonistic interactions that naturally occur at pasture. Two autochthonous and genetically related breeds (Aosta Chestnut and Aosta Pie Black) are involved in the battle contest, for which an exhaustive description has been given elsewhere (Mantovani *et al.*, 2007, Sartori and Mantovani, 2010). Briefly, the competitions consist of 20 preliminary tournaments per year in which the cows competing are divided into three weight categories and engage in duels wherein the winner is allowed to proceed further in the challenge and the loser leaves the contest. The battle board has no seeds and the most successful cows during the preliminary tournaments take part in the annual final competition. Each cow is allowed to compete in more than one preliminary tournament within a year. In the course of a tournament an individual can engage up to seven encounters depending on the number of participants (from 16 to 150 competitors within category).

The dataset analyzed in the present study was similar to that used in a previous study (Sartori and Mantovani, 2010), consisting of six successive years of battle contests (from 2001 to 2006). The raw data on dyad encounters were edited and organized to obtain for each cow within a given year-battle by category (YBxC): the number of wins or the final level of battle board reached (0 for final encounter, 1 for semi-final, etc.); the number of participants in each YBxC; the number of competitors for each cow in a YBxC and their identity in a progressive order from higher to lower level of the battle board. Other information recovered was the herds to which cows belonged at the moment of the battle, the age and weight at fight and the genealogical information for each cow. As compared to the previous dataset (Sartori and Mantovani, 2010), animals were allowed to enter the present dataset if their opponents were known and if they were recognized as opponents of other cows (i.e., some problems in data collection produced missing or wrong data). After this editing, a dataset of 15,273 individual records belonging to 5,236 cows was retained for all subsequent analysis. The total number of animals in the pedigree file recovered from the breeders' database was 12,476. Table 1 summarizes these and other descriptive statistics for the final dataset analyzed.

Table 1. Descriptive statistics on the final dataset from "Batailles de Reines" retained for analysis and relative to 369 year-battle x category levels (123 year-battle x 3 BW categories)

Item	Value
No. of records	15,067
No. of participants	5,236
No. of participants within year-battle x category	40.8 ± 23.5
No. of herd-year classes	2,337
No. of participants within herd-year	4.2 ± 3.4
No. of tournaments/cow	2.9 ± 2.4
No. of matches within tournament/cow	2.5 ± 1.4
No. of competitors/cow	1.9 ± 1.2
No. of animals in the pedigree file	12,476
Age of participant, years	6.2 ± 1.7
Body weight of participant, kg	549 ± 63
Body weight category, kg	
1 (heavy)	633 ± 44
2 (medium)	544 ± 20
3 (light)	494 ± 23

Phenotypes used

The main phenotypic record considered was the same as that analytically described in Sartori and Mantovani (2009 and 2010), where fighting ability was expressed as an individual placement score (PS) reflecting the position reached by the each participant as a function of the tournament size and the wins, differentiating the preliminary battles from the annual final match, but not accounting for the strength of the opponent. The PS (i.e., an example of social dominance index assessed through a competitive order as in Syme, 1974) could be expressed by the following formula:

$$PS_{ijkl} = 20 + ty_i + 2w_j + d_k, \quad (1)$$

where PS_{ijkl} is the score of cow l in a given tournament, depending on the type of tournament, ty_i (with ty = 0 for i elimination tournaments and ty = 7 for i final tournament in Aosta); on the number of wins (w_j) obtained by each animal in the given tournament category (with j = 0, …, 8); and on a tournament difficulty coefficient (d_k) related to the number of participants in the tournament category linked to the size of the battle board [5 classes, with k = −2 (>128 participants), −1 (65 to 128 participants), 0 (33 to 64 participants), 1 (17 to 32 participants), and 2 (<17 participants), respectively].

An alternative phenotype used in the present study was a scoring system aimed at considering the opponent strength; therefore, a competitive placement score (CPS, Sartori and Mantovani, 2009) was calculated accounting individually for the CPS of the last opponent of a cow, as expression of rivals' strength. In this case the CPS could be expressed as:

$$CPS_{ijkl} = 500 + ty_i - 2l_j + 2d_k - (500 - CPS_{ijkl'}), \quad (2)$$

where CPS_{ijkl} is the score of cow l in a tournament, depending on the type of tournament, ty_i (with ty = 0 for i heats and ty = 50 for i final tournament in Aosta); on the level of the battle achieved by each cow (l_j) in the given tournament category (with j = 0 for final, j = 1 for semi-final and so on up to j = 8); on a tournament difficulty coefficient (d_k) related to the number of participants and equal to that reported in [1]; and $CPS_{ijkl'}$ that represents the competitive placement score of the last opponent l' that cow l faced in the given tournament and category.

Models and comparison

The first step of the comparison was carried out among 4 different models considering: i) a classical quantitative genetic model expressing the fighting ability as PS and without contribution of the opponent (M1; Sartori and Mantovani, 2010); ii) a model considering CPS instead of PS, in order to account for the competitor strength directly in the phenotype (M2); iii) a model including the opponent as IGE and applying the PS as phenotype (M3); and iv) a model as in iii) but using CPS as phenotype (M4). Analyses of IGEs were carried out applying the competitive model as in Arango *et al.* (2005), originally implemented by Muir and Schinckel (2002). The "variance partitioning approach" of Bijma *et al.* (2007a) was adopted. Subsequently, a deeper investigation of the opponent contribution in the IGEs analysis was carried out through a different partitioning of the phenotypic variance following suggestions obtained from recent literatures (Arango *et al.*, 2005; Muir, 2005; Van Vleck and Cassady, 2005; Chen *et al.*, 2008; Wilson *et al.*, 2009). Six further models were therefore developed accounting differently for the permanent and associative components. The progressive complexity of models started from a simple repeatability model accounting only for the direct permanent environment of individual repeated measures (M5). The M5 model was then made more complex with the addition of the permanent environment provided by the conspecifics (M6), the further inclusion of the direct additive component (M7), followed by the associative variance component (M8). Aiming to check any possible shift of the permanent environmental variance, two further models considering either just the direct genetic variance (M9) or both direct and associative genetic components (M10) as random factors (i.e., without permanent environment) were considered. Table 2 gives an overview of the different models compared in the study.

Table 2. Overview of the different models, phenotype and variance components (other than the random residual) considered in the study.

Model	Phenotype[(1)]		Variance components[(2)]			
	PS	CPS	p_D	Σp_{Co}	a_D	Σa_{Co}
M1	√		√		√	
M2		√	√		√	
M3	√		√		√	√
M4		√	√		√	√
M5	√		√			
M6	√		√	√		
M7	√		√	√	√	
M8	√		√	√	√	√
M9	√				√	
M10	√				√	√

(1)PS is a placement score and CPS a competitive placement score accounting for the strength of the opponent directly at phenotypic level

(2)p_D is the random permanent environmental effect, Σp_{Co} is the random associative permanent environmental effect due to the interacting conspecifics, a_D is the random direct additive genetic effect, and Σa_{Co} is the associative genetic component (i.e., IGE).

With regard to fixed effects included in the ten different models, results of the preliminary ANOVA reported in Sartori and Mantovani (2010) were confirmed also in the present study. Therefore, the non-genetic fixed effects accounted for were: the day of tournament within weight category (YBxC or year-battle per category, 123 year-battles by 3 categories), the herd-year effect (HY, 2,429 levels), the age classes of cows (AC, 7 levels: ≤3, 4, 5, 6, 7, 8 and ≥9 years of age at fighting), and the individual weight as covariate within C.

In matrix notation, the most complete model adopted (i.e., M8) could be written as follows:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{W_D p_D} + \mathbf{W_C p_C} + \mathbf{Z_D a_D} + \mathbf{Z_C a_C} + \mathbf{e}$$

where $\mathbf{y}$ is an N×1 vector of observations, $\boldsymbol{\beta}$ is the vector of systematic fixed effects of order p, $\mathbf{p_D}$ is the vector of permanent environmental effects of order q when repeated phenotypes are used, $\mathbf{p_C}$ is the vector of permanent environmental effects provided by the conspecifics with the same order of $\mathbf{p_D}$, $\mathbf{a_D}$ is the vector of animal effects with order m, $\mathbf{a_C}$ is the vector of the associative variance component with the same order as $\mathbf{a_D}$, and $\mathbf{e}$ is the vector of residual effects. Furthermore, $\mathbf{X}$, $\mathbf{W_D}$, $\mathbf{W_C}$, $\mathbf{Z_D}$ and $\mathbf{Z_C}$ are the corresponding incidence matrices with the appropriate dimensions.

The assumptions about the structures of (co)variance in the most complete model were:

$$V\begin{bmatrix} a_D \\ a_C \\ p_D \\ p_C \\ e \end{bmatrix} = \begin{bmatrix} A\sigma^2_{a_D} & 0 & 0 & 0 & 0 \\ 0 & A\sigma^2_{a_C} & 0 & 0 & 0 \\ 0 & 0 & I\sigma^2_{p_D} & 0 & 0 \\ 0 & 0 & 0 & I\sigma^2_{p_C} & 0 \\ 0 & 0 & 0 & 0 & I\sigma^2_e \end{bmatrix},$$

where $\sigma^2_{a_D}$ is the direct additive genetic variance, $\sigma^2_{a_C}$ the associative genetic variance, $\sigma^2_{p_D}$ the direct permanent environmental variance, $\sigma^2_{p_C}$ the permanent environmental variance due to conspecifics, σ^2_e the residual variance, $\mathbf{A}$ the numerator relationship matrix and $\mathbf{I}$ an identity matrix. It should be noted that due to the absence of a close relationship among animals the off diagonals were not taken into account and in all models where conspecifics effects were considered, both Vp_C and Va_C were obtained as described by Arango *et al.* (2005) multiplying the estimated component by the average number of competitors (n=1.9; Table1).

All analyses have been carried out applying the EM-REML method (expectation maximization-restricted maximum likelihood) to a single trait animal model using the appropriate program of the BLUPF90 family as software (Misztal, 2008).

Models were compared using the Akaike Information Criterion (AIC; Akaike, 1974), and the Bayesian Information Criterion (BIC; Schwarz, 1978). Additional comparisons had been carried on using PSB (Percentage Square Biases (Ali and Schaeffer 1987) and MAD (Mean Absolute Deviation (Schildcrout and Heagerty 2005) methods. However, we decided to retain at the en only AIC and BIC as the most informative techniques.

Heritability estimates and standard error

Depending on the variance components estimated, different heritability (h^2) values were calculated and compared for the ten models analyzed. When direct additive variance was the only estimate, a direct h^2 was obtained by dividing Va_D by the sum of all other variance components. In the same way, associative genetic variance was computed as the ratio between Va_C and all the other variances. When both direct Va_D and associative Va_C variance components were accounted in the model, both direct h^2_D, associative h^2_C and total heritability ($h^2 = h^2_D + h^2_C$) were obtained. Standard errors (SE) for heritability estimates were approximated using the formula of Falconer (1989):

$$SE_{\hat{h}^2} = 4\sqrt{\frac{2(1-t)^2[1+(k-1)t]^2}{k(k-1)(s-1)}}$$

where t is the intraclass correlation approximated by ($h^2/4$) for paternal half-sib estimates, k is the mean number of offspring per sire, and s is the number of sires obtained from the pedigree file.

Repeatability (r) and the evolvability (CV_A, expressed by the coefficient of additive genetic variation; Houle, 1992) were also obtained. Repeatability, as always, considered all estimated variance components at numerator and all components plus the residual at denominator. On the other hand, evolvability was computed as:

$$CV_A = 100\sqrt{\sigma_a^2}\Big/\overline{X},$$

where CV_A includes both direct and associative genetic variance when assessed and $\overline{X}$ is the average fighting ability phenotype.

Spearman rank-order correlations using the proc CORR (SAS Institute, 2004) between EBVs obtained with different models were also obtained for model comparison.

Table 3. Model fitting parameters (x 10^3), variance components and parameters estimates in different models

Model	Fitting[†]		Variance Components[‡]					Genetic parameters					
	AIC	BIC	V_{pD}	V_{pC}	V_{aD}	V_{aC}	V_e	h^2	h^2_D	h^2_C	SE h^2	r	CV_A
M1	69.86	69.88	1.26		0.59		5.91	0.076	0.076		0.042	0.239	3.335
M2	124.9	125.0	86.20		13.50		564.0	0.020	0.020		0.040	0.150	0.783
M3	34.86	34.89	0.03		0.01	0.14	1.49	0.091	0.008	0.083	0.043	0.107	1.688
M4	102.2	102.3	20.17		2.85	0.55	377.0	0.008	0.007	0.001	0.040	0.059	0.393
M5	64.45	64.46	1.66				5.99					0.217	
M6	45.81	45.84	0.10	7.40			1.45					0.838	
M7	51.81	51.84	0.04	7.40	0.06		1.46	0.006	0.006		0.040	0.837	1.015
M8	25.20	25.23	0.03	0.15	0.01	0.01	1.46	0.012	0.008	0.004	0.040	0.119	0.621
M9	69.92	69.93			1.95		6.02	0.245	0.245		0.047	0.245	6.055
M10	34.86	34.88			0.03	0.18	1.50	0.100	0.018	0.082	0.043	0.100	1.772

[†] AIC is the Akaike Information Criterion (Akaike, 1974), and the BIC the Bayesian Information Criterion (Schwarz, 1978)

[‡] V_{pD} is direct permanent environmental variance, V_{pC} is the associative permanent environmental variance, V_{aD} is the direct additive genetic variance, V_{aC} is the associative additive genetic variance and V_e is the residual variance.

h^2 is the overall heritability, h^2_D is the direct heritability and h^2_C is the associative heritability, SE h^2 is the standard error of heritability (Falconer, 1989):, r is the repeatability and CV_A is the evolvability, or the coefficient of additive genetic variation (Houle, 1992)

RESULTS

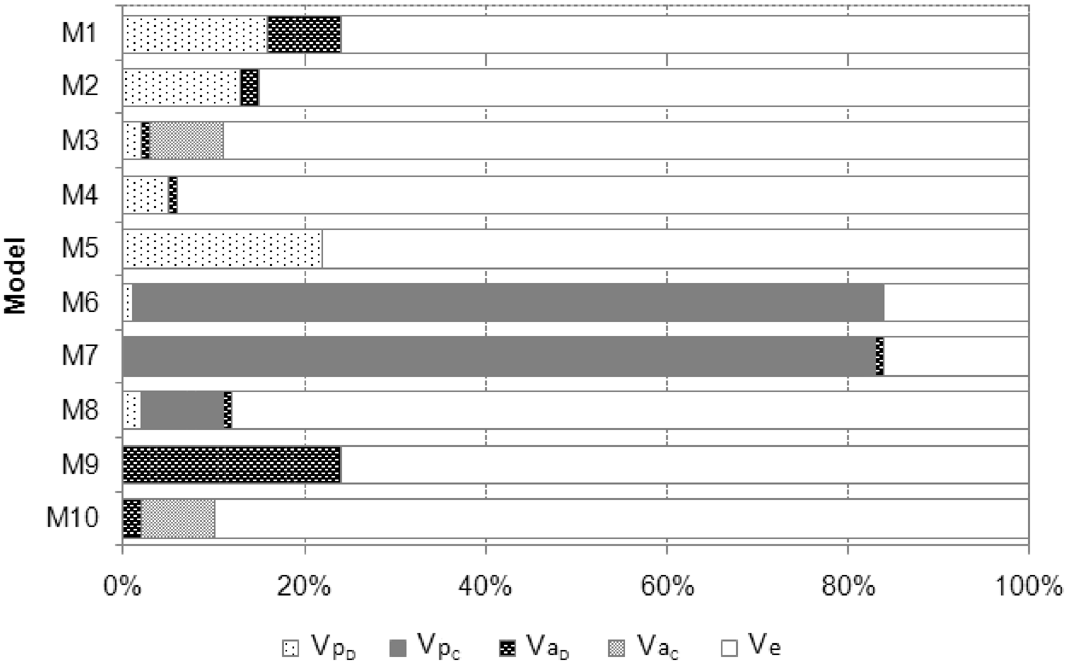
Model comparison

The goodness of fit statistics derived from the likelihood function (Table 3), indicates a wide variability for both AIC and BIC values, which ranged from 124,987 (M2), to 25,196 (M8) and from 125,010 (M2), to 25,234 (M8), respectively. The rank of different models with regard to both AIC and BIC parameters was almost similar, with lowest values, i.e., better fitting, for the model with the highest number of variance components to be estimated. With the only exception of M2 and M4, i.e., models based on CPS, that always showed the worst fitting, the number of estimated variance components influenced the AIC and BIC values. When the PS was used as phenotype, the introduction of the competitor effect as IGE led to a better model fitting than using direct permanent and/or additive variance components only. As a matter of fact, including both associative environmental and genetic factors led to the lowest values of AIC and BIC (M8); on the other hand, avoiding any IGE with PS phenotype (M5, M1 and M9), resulted in the worst (i.e., highest) AIC and BIC.

Estimated variance components

Direct permanent environmental and genetic variances estimated with M1 were respectively 16% and 8% of the total phenotypic variance (Table 3 and Figure 1), leading to a heritability of 0.076 and a repeatability that was threefold higher (i.e., 0.239; Table 3). Heritability and repeatability of the fighting ability noticeably decreased in the model including the same variance components but considering the conspecifics within the phenotype (M2), reaching the values of 0.020 and 0.150, respectively. This model revealed an increase in the residual variance estimates as compared to M1 (from 76% to 85%; Figure 1). When conspecifics were included in the model as associative genetic component (i.e., IGEs, M3 and M4) an even greater increase of the residual variance was observed, (89 and 94% for M3 and M4, respectively; Figure 1), associated with a reduction in the permanent environmental variance (2 and 5% for M3 and M4, respectively; Figure 1), that led to an estimated heritability of 0.091 and 0.008, for M3 and M4, respectively (Table 3). The conspecific contribution in M3 was eight-fold greater than the direct additive component, (i.e., 8% of the total variability; Figure 1), whereas In M4 it was nil. When permanent environmental or additive genetic

Figure 1. Partitioning of variances estimated with ten different models considered in the study.



V_{pD} is direct permanent environmental variance, V_{pC} is the associative permanent environmental variance, V_{aD} is the direct additive genetic variance, V_{aC} is the associative additive genetic variance and V_e is the residual variance.

components were estimated alone (M5 and M9), they accounted for almost all the variance previously partitioned between direct additive genetic and permanent environment as in M1, with an incidence of residual variance similar to that obtained in M1 (78 vs. 76 vs. 76% for M5, M9, and M1, respectively, Figure 1). However, in M9 the genetic component was inflated, as can be seen in the heritability value that reached 0.245 (Table 3). When accounting for an indirect permanent environmental component without IGE (M6 and M7), there was a shift of a notable part of the variance to the associative component, gathering 83% of the whole and leaving only 16% for the residual variance (Figure 1). The main difference between M6 and M7 was a shift of 1% of the direct environmental variance to the direct genetic component, leading to a heritability of only 0.006 in M7 (Table 3). Due to such great influence from the permanent environment, the repeatability for both M6 and M7 was about 0.84, i.e. the greatest values obtained. The introduction of the IGE component in the most complete model (M8), produced a reduction of all estimated variances, with an incidence of

2% and 9% for direct and associative environment, respectively (Figure 1), and only 1% of incidence for the direct genetic component. As a consequence, total heritability reached the very small value of 0.012, with a repeatability of 0.119 (Table 3). Considering M10, i.e., a model with only genetic components, the heritability was close to that in M1 and M3 (Table 3), due to the shift of the permanent component to the residual variance. Standard errors of the heritability estimates were similar across the models, ranging from 0.040 to 0.047 (Table 3). With regard to the coefficient of variation of additive variance (i.e., CV_A), the general low values of heritability estimates was also associated with low evolvability values. The lowest evolvabilities were for M8 and M4 (Table 3), i.e., models with lowest incidence of genetic variance. On the other hand, models with the greatest incidence of genetic variance (M1 and M9) showed the greatest evolvability value (Table 3).

Correlations among Estimated Breeding Values (EBVs)

A strong correlation existed within the same type of model (i.e., accounting for IGEs or not; M1 vs. M2 and M3 vs. M4) when the same phenotype was used, i.e. PS for M1 and M2, and CPS for M3 and M4, respectively (Table 4). On the other hand, rankings between estimated breeding values (EVBs) proved to be low to moderate (from 0.435 to 0.523; Table 4) when models were complicated adding the associative genetic component (IGE) as in M3 and M4 as compared to M1 and M2 (i.e., values may be affected by the low levels of heritability). With regard to other comparisons carried out considering only the PS as phenotype, the greatest coefficient of correlations were obtained comparing M1 and M9 (0.996; Table 4), i.e., models that did not consider any associative component, and comparing M3, M8 and M10 (from 0.996 to 0.999; Table 4), i.e., within models that accounted for all direct and associative genetic components. Like the previous across model analysis, the comparison among models that differed for the presence or not of a associative components, produced low coefficients of correlation between EBVs, indicating great changes in the ranking of animals (Table 4). In particular, the greatest changes occurred comparing EBVs from M3 with EBVs from M7 (0.316 of correlation) or comparing M7 with M10 (0.322 of correlation). In both cases, the IGE was changed with an associative permanent environmental component. However, also the comparison of EBVs from M7 and M8 produced a 0.331 coefficient of correlation, due in this case to the inclusion of both IGEs and the associative permanent environmental component.

Table 4. Spearman rank-order correlation coefficients among EBVs obtained with some of the different model compared in the study (all coefficients were significantly different from 0 for $P < 0.001$)

	Model						
Model:	M2	M3	M4	M7	M8	M9	M10
M1	0.823	0.524	0.482	0.600	0.524	0.924	0.532
M2		0.435	0.470	-	-	-	-
M3			0.962	0.316	0.997	0.615	0.999
M4					-	-	-
M7					0.331	0.554	0.322
M8						0.615	0.996
M9							0.623

DISCUSSION

The first part of this study was aimed at evaluating social dominance comparing genetic models without the opponent (M1) to models that accounted for the competitors either within the phenotype (M2) or within the model (M3), i.e., as IGEs. The comparisons of estimated variance components and model fitting statistics confirmed the results of Sartori and Mantovani (2009), where the introduction of the competitor in the score (i.e., Competitive Placement Score or CPS) provided a worse fit to the data compared to the classical quantitative models and models with IGEs. This could be due to the remarkable rise of phenotypic variability introduced by accounting for the opponent, increasing the residual variance compared to the genetic and permanent components. Goodness of fit criteria (i.e., AIC and BIC) indicated that including the opponent within the model as IGE (i.e., M3) provided a better model fit. On the other hand, the M4 model aimed to detect the magnitude of the opponents effect when included both as phenotype and IGEs, underestimates the genetic competitor effect, as the low associative genetic variance indicates. As indicated by some recent literature accounting for IGEs (Arango *et al.*, 2005; Van Vleck *et al.*, 2007), comparisons carried out on M1-M4 lack the associative permanent environmental component due to the opponent. This was introduced in subsequent models following the suggestions given by Wilson *et al.* (2009). The introduction of the associative environment in M6 and M7 exaggeratedly inflated the permanent environmental variance. Accounting for associative genetics in M8 brought the explained variance back to more reliable values, although the magnitude of the associative component proved to be low, as in the estimated heritability, in spite of a consistency with literature on cow fighting ability (Plusquellec, 2001). It is important to note that the overestimation of the permanent environment effect observed with M6 and M7 due to the introduction of an associative component, could also be reflected in M8, with a coverage of some part of associative genetic variance. Recent literature investigating IGEs in social behaviors generally found similar or greater associative genetic variances compared to the direct component (Arango et al., 2005; Wilson et al., 2009). As a consequence, this model could be considered the touchstone for genetic estimation of fighting ability in cattle thanks also to a demonstrated better model fitting. However, in a recent work of Bijma and Wade (2008) on average daily gain in pigs, problems in assessing associative permanent environmental components have been reported. Such problems could be related to associative components that may introduce hidden variability, i.e., not captured by the single phenotype, but that need to be considered through the whole interaction within a group. Indeed, the inclusion of the pen and of the litter of conspecifics as random environment into the recent genetic models based on the partition of variance and developed for livestock (Van Vleck and Cassady, 2005; Bergsma et al., 2008), attests the great weight of the group of conspecifics as a source of variation. In such a perspective, a suitable alternative could be the model accounting for IGEs but not for the associative permanent environment (i.e., M3), exhibiting a good fitting and quotes of variance consistent with literature. Moreover, similar values of heritability estimates have been already found for fighting ability in Heréns cattle (Plusquellec, 2001), closely related to Aosta Chestnut and Black Pie cattle. The recent wave of applied studies investigating social traits in livestock has provided important contributions both in theoretical concerns (i.e., the relatedness of interacting conspecifics, Cheng et al., 2009) and in operative examples (i.e., survival in hens, Ellen et al., 2008). All these studies followed the partitioning variance approach proposed by Bijma et al. (2007b), also applied in our current study. Due to their economic relevance, the most analyzed traits have been the average daily gain, widely studied in pigs (Chen et al., 2008), in bulls (Van Vleck et al., 2007), chicks (Van der Waaij et al., 2010) and cod (*Gadus morhua*; Monsen et al., 2010), and survival, a crucial factor in poultry and pig farms (Muir, 2005; Roehe et al., 2010). Some other studies also focused on traits with indirect economic relevance, such as infectious diseases (Lipschutz-Powell et al., 2010). All these studies involved non-behavioral traits affected by the social contest, that can be included in the first of the three types of interacting phenotypes that Moore et al. (1997) theorized. In such type, only one trait is affected by the interaction. On the other hand, social dominance and therefore fighting ability may mostly be considered as a second type interaction (i.e., a trait in the focal individual that influences another trait in the opponent and vice versa), since the dominant postures of a contestant induces submissive postures in the opponent (Moore et al., 1997). In this perspective, accounting for just one phenotype as the sum of all the interactions occurring among two conspecifics, as in the present study, could lead to problems in identifying the respective genetic contributions of focal individual and interacting partner. On the other hand, partitioning social dominance into several components could lead to underestimation or overestimation of the variance components involved, as well as problems in data collection and analysis which could arise from the huge amount of data required. A partitioning variance based approach for assessing social traits related to dominance has recently been carried out by Wilson et al. (2009), whereas the dissertation of Moore et al. (2002) and the relative operative example based on cockroaches (*Nauphoeta cinerea*) followed the approach of interacting phenotypes. Such an approach has shown itself useful in genetic investigations for complex social interactions arising from the contribution of many different traits, such as courtship (Miller and Moore, 2007; Chenoweth et al., 2010; Snook et al., 2010) and cooperation (Charmantier et al., 2007; Bijma and Wade, 2008). All these studies provided important evolutionary explanations of social dynamics accounting for IGEs, expanding the first dissertations of heritability estimations and evolutionary changes involving interacting phenotypes described by Wolf et al. (1998). Concerning social dominance, the current study has quantified a low heritability of the trait but consistent with the other behavioral traits investigated either with the classical quantitative models (Mosseau and Roff, 1987) or IGEs approaches (Wilson et al., 2009). Thus, a response to selection in terms of evolvability (i.e., coefficient of additive variation), also proved to be achievable in an IGE model, as already carried out for sexual conflict (Moore and Pizzari, 2005). Social dominance exhibits a general low evolvability, but it was probably due to the strong magnitude of environmental incidence.

A remarkable contribution in genetic studies investigating such a complex scenario can be provided by the already mentioned artificial selection carried out on livestock, thanks to the large population sizes, the complex pedigrees and the richness of information about individuals that are generally available. In this perspective, the current study of cow fighting ability even attests how resources coming from animal breeding can be a powerful tool in investigating a crucial aspect of living in groups, as is social dominance. Moreover, concerning the fighting behaviour in Aosta Chestnut and Pie Black cattle, a well-performed selection for social dominance may avoid undesirable effects such as decreased milk production or reduced fertility that could follow a selection for masculine traits related to agonistic activities, such as aggressiveness or increased body size. Actually, accounting for IGEs in animal breeding allows selection to move away from the customary genetic improvement when necessary, leading to results that can prove to be crucial in terms of animal welfare.

CONCLUSIONS

Social dominance has recently been studied in groups of cattle through the assessment of fighting ability during traditional competitions of Aosta Chestnut and Pie Black cows. Thus, an investigation of the role of the conspecifics in the genetic inference of such social traits has been possible, and the comparison performed between models accounting for the opponents either in the phenotype or within the model as indirect genetic effects has attested that the best estimations are obtained when the social partner is directly included within the genetic model. Moreover, both associative genetics and associative environment play a role as contributors of conspecifics, but the introduction of the latter within the model leads to overestimations of permanent environmental variance. Finally, the analyses performed shed light on a level of heritability for fighting ability that is low, but sufficient for driving evolutionary changes.

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Chapter 4

Running head: Inbreeding effects on fighting ability

EFFECTS OF INBREEDING ON FIGHTING ABILITY MEASURED IN AOSTA CHESTNUT AND AOSTA BLACK PIED CATTLE

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In submission

"The harmful effects of close inbreeding
have been noticed for many centuries"
(Charlesworth and Charlesworth, 1987)

ABSTRACT: - Aosta Black Pied (ABP) and Aosta Chestnut (AC) are dual-purpose cattle autochthonous of West Alps and famous for a strong temperament revived in traditional competitions. The present study aimed to investigate the influence of inbreeding on breeding values for fighting ability achieved on participants. Data (n=23,998) of 8,259 cows competing in years 2001-2009 were analysed to find out breeding values for the trait. A placement score was chosen as phenotype for fighting ability and both a classical quantitative model (M1) and its implementation accounting also for indirect genetic effects (M2) were analyzed using EN-REML method. A heritability of about 8% was thus estimated via REML method, and positive variations of the breeding values (EBVs) over years (+2.1%/year for ABP and +3.1%/year for AC) were observed despite a lack of selection for fighting ability. A genetic analysis carried on pedigree data of all animals born in years 1990-2009 (19,554 and 87,967 records for ABP and AC, respectively) showed an increase of inbreeding over years. The trend resulted greater for ABP (+0.06%/year) than for AC (+0.03%/year) populations, that presented also a grater mean inbreeding (F=2.96±0.80% vs. 1.01±1.02% for ABP and AC, respectively) and mean average relatedness among individuals (AR=1.22±1.02% vs. AR=0.43±0.29% for ABP and AC, respectively; data referring to 2009 born). Including alternatively F or AR within the genetic model the heritability estimates for FA did not change, although they were both significant in the preliminary ANOVA. Moreover, linear analyses carried on the 33 major lineages to which most of participants belonged (n=6,087) revealed an overall negative trend of EBVs as respect to the increment of either F (b = -21.3, $P<0.01$) or AR (b = -50.1, $P=0.001$). Hence, there seems to be an inbreeding depression also in the behavioural trait of both ABP and AC cattle and its knowledge should be relevant for breeding management.

Key words: Fighting Ability, Inbreeding, Average Relatedness, Quantitative genetics

INTRODUCTION

Inbreeding is the result of mating among relatives which is associated to an increase in homozygosity (Jacquard, 1975). It can be quantified by the coefficient of inbreeding F, defined as the probability that two alleles at any locus in an individual are identical by descent to a base population (Wright, 1922; Malécot, 1948; Falconer and Mackay, 1996; Lynch and Walsh, 1998). Inbreeding has usually deleterious effects on offspring fitness, leading to a reduction in the mean phenotypic performances (Falconer and Mackay, 1996). This depression due to inbreeding may be mainly accounted for two different causes reviewed by Wright (1977): 1) the overdominance hypothesis, explaining that heterozygotes loci are superior in fitness than the homozygotes, and 2) the dominance hypothesis, assuming recessive or partially recessive deleterious effectd of alleles in homozygosis condition.

Assortative mating among relatives may occur both in the wild, primarily when a reduction in population size happens, and in domesticated animals, where selection is routinely applied and only the best-selected individuals are allowed to mate (Kristensen and Sørensen, 2005). Negative effects of inbreeding have been well documented in nature, as well as in laboratory experiments and in animal and plant breeding practises (Charlesworth and Charlesworth, 1987; Crnokrak and Roff, 1999; Kristensen and Sørensen, 2005). The magnitude of inbreeding depression varies among populations, species, settings and traits (Keller and Waller, 2002), and even the presence of diverse founders may affect inbreeding coefficients in lineages of offspring (Miglior et al., 1994; Lacy et al., 1996). Inbreeding effects commonly reveal to be greater in traits related to fitness (life-history traits; i.e., viability, survival, fecundity, longevity) than in morphological traits, as body weight or muscularity, that are less involved in fitness (Falconer and Mackay, 1996; DeRose and Roff, 1999). Examples of life-history and morphological traits affected by inbreeding have been accounted both in wild and selected populations (i.e., Keller, 1998; Slate et al., 2000; Sewalem et al., 2006; McParland et al., 2008), and they have been estimated either through quantitative analysis on long-term pedigrees or measuring internal relatedness by genetic markers (Keller and Waller, 2002). Some evidences of inbreeding depression have been recognized also for behaviour, in particular within traits under sexual selection as male courtship or female choice, that are closely related to fitness (Cheng et al., 1985; Ala-Honkola et al., 2009; Bohlund et al., 2010). Incidence of inbreeding varies within different behaviours, revealing as small or lacking on traits such as temperament (Burrow, 1998; McParland et al., 2007), or noteworthy on traits that are relevant in disputes over resources, such as aggressiveness or fighting ability (Tiira et al., 2003; Välimäki et al., 2007). Effects of mating among relatives on fighting ability have been demonstrated both in laboratory and field studies (Eklund 1996; Hoffman et al., 2004; Välimäki et al., 2007), but not in livestock. Concerning cattle, negative inbreeding effects have been already observed for milk production, fertility, survival, growth traits and temperament (i.e., Burrow, 1998; Smith et al., 1998; Sewalem et al., 2006; McParland et al., 2007).

The recent studies on breeding values for fighting ability carried on fighting cattle breeds (i.e., Aosta Chestnut and Aosta Black Pied; Mantovani et al., 2007; Sartori and Mantovani, 2010), could represent a interesting sources of data for investigating the effects of inbreeding on competitiveness in a livestock species.

Thus, the present study has aimed to evaluate: 1) the level of inbreeding in Aosta Chestnut and Pie Black cattle population, and 2) the incidence of inbreeding level on fighting ability within the same population. As outcome of this study, trends of both inbreeding and fighting ability have been taken into account and discussed.

MATERIALS AND METHODS

Data of fighting performances have been attained following the guidelines that the farmer association responsible for the battle organization provided. These guidelines are in agreement with the Italian legislation on animal care.

Subject of the study

Aosta Black Pied (ABP) and Aosta Chestnut (AC) are rustic cattle autochthonous of Italian Western Alps and constituting, together with the Aosta Red Pied, the “Valdostana Breeds”. Managed into the same herd book, ABP and AC are separately bred in order to preserve the main traits of the population and the typical coat colour, spotted black and white in ABP, and shading from dark chestnut into bright in AC. The two varieties seem also to have different origins: ABP can be ascribed to an Illyrian population coming from North European (FAO, 2011), whereas AC may be traced back to a group of Swiss Heréns cattle that crossed Alps (Del Bo et al., 2001). The two varieties constitute, together with the local Aosta Red Pied cattle and the Swiss breeds Heréns and Evolene, a monophyletic group of Western Alps populations (Dal Bo et al., 2001). The two ABP and AC breeds exhibit different population sizes, as herd book told in 2010: a total of 22,857 individuals, made up by 11,958 cows, 147 bulls and 10,752 calves were ascribed to AC population, whereas ABP comprehended 1,394 individuals, including 745 cows, 645 calves and only 4 bulls. Both breeds are not endangered (FAO, 2011), but the scarce number of individuals in ABP needs to be monitored. The breed suffered a strong reduction of the population in the 1960s, due to the substitution by more productive cosmopolitan breeds. On the other hand, AC has received noteworthy contribution from Heréns lineage over time, in order to enhance the peculiar attitude to fight exhibited in traditional competitions. Both AC and ABP have been involved for centuries in manifestations called “Batailles de Reines”, aimed to revive the hierarchical disputes naturally occurring at pasture. Thus, hundreds of cows per day are carried into a grass arena and engage knock-out battles in couples contending the title of “Queen of the year”. A detailed description of traditional cow battles has been already provided (Sartori and Mantovani, 2010). Briefly, 21 days of competitions take place every years, and participants are involved in three contemporary tournaments by weight category. Cows are accepted into more than one tournament per year, although only pregnant cows living in Aosta region and belonging only to the two ABP and AC populations are allowed to participate. Despite the strong interest that both farmers and tourists have on battles, selection for fighting ability has been only empirical till now. A study started in 2007 (Mantovani et al., 2007) and carried out over years (Sartori and Mantovani 2009, Sartori and Mantovani, 2010), permitted to assign a phenotypic value for fighting ability and to estimate genetic parameters and merit indexes. Now, the introduction of fighting ability within the aggregate genetic index of the breed currently comprehending the two genetic indexes of milk and muscularity is incoming.

Data description

Data belonging to nine subsequent years of battles (2001-2009) were collected by the farmer association organizing the challenge (*Amis de Reines*) and thus provided by the regional farmer associations (AREV). Additional information about pedigree and herds came from the national breeder association (ANABoRaVa) and the regional farmer association, respectively. Raw data were edited as in Sartori and Mantovani (2010), and an amount of 23,998 records belonging to 8,259 participants and related to 17,224 animals in the pedigree was retained for the further analyses. Among participants, 791 cows belonged to ABP population, whereas AC accounted for 7,468 members. A total of 79 founders (i.e., animals without any known ancestors and each one precursor of distinct lineages) have been identified for the 6,245 participants possessing at least one known parent. Descriptive statistics about battle dataset are provided in Table 1.

Pedigree information on 27,638 ABP and 106,061 AC individuals were extracted from the Valdostana breeds herd book. Purebred animals were defined as individuals with both parents as purebreds, and they were recognized as the 98,36% of the considered ABP population and 98,86% of the AC members. Data were available since 1960 and since 1961 for AB and AC, respectively. Pedigree information are available in Table 2.

TABLE 1. Descriptive statistics on the final dataset from “Batailles de Reines” retained for analysis and related to 558 year-battle x category levels.

Item	Value
No. of records	23,998
No. of animals in the pedigree file	17,224
No. of participants	8,259
No. of Aosta Black Pied cattle	791
No. of Aosta Chestnut cattle	7,468
No. of known fathers of participants	1,156
No. of participants within year-battle x category	43.0 ± 23.5
No. of herd-year classes	3,667
No. of participants within herd-year	6.5 ± 6.3
No. of founders	79
No. of participants with founder (i.e., known parents)	6,245
No. of participants ascribing to the same founders	79.1 ± 175.6
No. of tournaments/cow	2.9 ± 2.5
No. of matches within tournament/cow	2.5 ± 1.4
No. of competitors/cow	1.9 ± 1.2
Age of participant, years	5.8 ± 1.5
Body weight (BW) of participant, kg	553 ± 63
1st BW category (heavy), kg	639 ± 44
2 BW category (medium), kg	547 ± 20
3 BW category (light), kg	497 ± 22

TABLE 2. Pedigree information of Aosta Black Pied and Aosta Chestnut cattle and data retained for population analyses. Pedigree referred to August, 2010 update.

Whole pedigree: 133,699 individuals	Aosta Black Pied (ABP)	Aosta Chestnut (AC)
Individuals retained for analysis	27,638	106,061
Purebred individuals (i.e., both parents belonging to the same breed)	27,184	104,854
-males	*7,764*	*42,737*
-females	*19,420*	*62,117*
Year from which data are available	1960	1961
Year from which individuals are inbred	1978	1986
Average animals per birth year[a]	4,398±1,266	4,398±1,266
-males	*1,997±951*	*1,997±951*
-females	*2,402±340*	*2,402±340*

[a]Referred to individuals born in years 1990-2009

Statistical analyses

Phenotypic values for fighting ability have been assessed as in Sartori and Mantovani (2010), assigning an individual placement score (PS) to the performance that a cow had in a tournament. PS could be expressed by the subsequent formula:

$$PS_{ijkl}=20+ty_i+2w_j+d_k \qquad (1)$$

where PS_{ijkl} is the score of cow l in a given tournament, due to: the type of tournament ty_i (with ty=0 for i=eliminatory tournaments and ty=7 for i=final competition), the number of wins w_j that an animal obtained in the tournament-category it fought (j=0,…, 8) and a difficulty coefficient d_k related to the battle-board size (5 classes with k=-2: >128 participants, -1: 65-128, 0: 33-64, 1: 17-32 and 2: <17 participants, respectively).

Individual inbreeding coefficients (F) were calculated through a recursive algorithm written in Microsoft Fortran language (Microsoft Corporation, 1994) and assigning the yearly average inbreeding coefficients computed in the previous iteration as starting values for missing data. Individual inbreeding coefficients revealed highly correlated (r=0.93, $P<0.001$; Pearson correlation, CORR procedure; SAS Institute, 2004) with the same estimates carried out through an alternative approach (Gutiérrez and Goyache 2005). This second way permitted to assess individual coefficients of average relatedness (AR; Goyache et al., 2003), expressing the contribution of an individual within the pedigree as the probability that an allele chosen from the whole population by chance belongs to that target animal. The mean inbreeding coefficient of a population is a common measure of its level of homozygosis. Average kinship coefficients (i.e., F and AR) have been estimated for participants at battles as well as for the whole population (i.e., data belonging to the last twenty years, 1990-2009). Moreover, average estimates were also assessed by sex and breeds. In order to quantify the similarity among the kinship parameters, a Pearson product-moment correlation analysis (Correlation procedure; SAS Institute, 2004) was performed among F and AR.

Aiming to investigate the eventual relations among fighting ability and inbreeding, the following analyses have been carried out: i) a genetic evaluation for fighting ability accounting for inbreeding coefficients or not; ii) an assessment of the genetic trend on individual values (EBVs) for fighting ability between participants to battles; iii) an estimation of the yearly trend for inbreeding coefficient realized on the whole pedigree for both Aosta Black Pied or Aosta Chestnut; iv) an investigation of how EBVs for fighting ability vary in relation to inbreeding and among lineages of different founders.

i) Genetic evaluation for fighting ability accounting for F or not

The investigation was carried out among different models accounting for inbreeding coefficients or not; as starting models, a classical quantitative genetic model (M1) was built following Sartori and Mantovani (2010), and a genetic model (M2) including an associative genetic variance as indirect genetic effect (IGE; Moore et al., 1997; Arango et al., 2005; Bijma et al., 2007) was developed from M1. This model corresponded to one of the models (i.e., M3) on which a recent work aimed to investigate IGEs on fighting ability has reasoned about (Sartori and Mantovani, *in submission*). As random effects, both of the models included the permanent environment due to the repeated measurements on individuals arising from different battles and a direct additive genetic component came up from pedigree, as well as the residual variance. Following Sartori and Mantovani (2010), fixed effects accounted for were: the day of tournament within weight category (YBxC or year-battle per category, 558 levels ascribing to 3 weight categories), the herd-year (HY, 3,667 levels), the ages of cows, divided in classes (AC, 7 levels: ≤3, 4, 5, 6, 7, 8 and ≥9 years of age at fights), and the individual weight as covariate within C. The two models (i.e., M1 and M2) were thus implemented with the inbreeding coefficient F alternatively considered as (M3 ad M4) covariate within breed (d_o*$F_{m:o}$; 2 levels of d, corresponding to the two ABP and AC), or (M5 and M6) grouped into 7 classes (Fc_n, with Fc=0, 0<Fc<3.125, 3.125≤Fc<6.25, 6.25≤Fc<12.5, 12.5≤Fc<18.25, 18.25≤Fc<25.0, and Fc≥25.0%) as in other analogous studies (i.e., Sewalem et al., 2006). Preliminary analyses of variance (ANOVAs; GLM procedure; SAS Institute, 2004) were carried on the fixed factors either considered without inbreeding coefficient, or including F alternatively as covariate by breed or as class (see above).

The most complete model adopted (i.e., accounting for indirect genetic effects and for inbreeding coefficient) could be written via matrix notation as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{W}\mathbf{p} + \mathbf{Z_D}\mathbf{a_D} + \mathbf{Z_C}\mathbf{a_C} + \mathbf{e} \qquad (2)$$

where **y** is an N×1 vector of observations, **β** is the vector of systematic fixed factors of order p, **p** is the vector of permanent environmental factors of order q due to the repeated phenotypes, $\mathbf{a_D}$ is the vector of animal effects with order m (i.e., direct additive genetic variance), $\mathbf{a_C}$ is the vector of the associative variance component with the same order as $\mathbf{a_D}$, and **e** is the vector of residual effects. Furthermore, **X**, **W**, $\mathbf{Z_D}$ and $\mathbf{Z_C}$ are the related incidence matrices with the proper dimensions. The resulting assumptions about the structures of (co)variance were:

$$V\begin{bmatrix} a_D \\ a_C \\ p \\ e \end{bmatrix} = \begin{bmatrix} A\sigma^2_{a_D} & 0 & 0 & 0 \\ 0 & A\sigma^2_{a_C} & 0 & 0 \\ 0 & 0 & I\sigma^2_p & 0 \\ 0 & 0 & 0 & I\sigma^2_e \end{bmatrix},$$

where $\sigma^2_{a_D}$ is the direct additive genetic variance, $\sigma^2_{a_C}$ the associative genetic variance, σ^2_p the permanent environmental variance (i.e., direct random component), σ^2_e the residual variance, **A** the numerator relationship matrix and **I** an identity matrix. The absence of close relationships (i.e., full-sibs or half-sibs conspecifics) justified the lack of off diagonals-values, whereas $\sigma^2_{a_C}$, when considered, was obtained multiplying the assessed component by the average number of competitors (n=1.9; Table 5), as in Arango *et al.* (2005). Pearson and Spearman correlations (Correlation Procedure; SAS Institute, 2004) were performed among the estimated variance components by type of model (i.e., either classical quantitative model or model also accounting for IGEs). This analysis was carried out in reason to quantify the eventual differences in genetic estimations due to the introduction of the inbreeding coefficient. A further model (M7) was developed from the classic quantitative one (i.e., M1) introducing, instead of F, the average relatedness of participants covariate by breed (d_o*$AR_{m:o}$) as alternative measure of individual homozigosity. A preliminary ANOVA was carried out also including AR among the fixed factors. An overview of all considered models is offered in Table 3. Genetic analyses were performed using the EM-REML method (expectation maximization-restricted maximum likelihood) that the BLUPF90 family of software (Misztal, 2008) provided. Models accounting for IGEs were written following the "variance partitioning approach" of Bijma *et al.* (2007) and analyzed through a competitive model as illustrated in Arango et al. (2005). The Akaike Information Criterion (AIC; Akaike, 1974) was thus applied in order to compare the diverse models.

TABLE 3. Overview of the diverse models, phenotype, fixed and random factors (i.e., variance components) considered in the study.

	Phenotype	Fixed factors [b]								Random factors [c]			
	y_{ijklmn}	μ	YB_i*C_j	HY_k	AC_l	b_j*$W_{m:j}$	d_o*$F_{m:o}$	Fc_n	d_o*$AR_{m:o}$	p_m	a_n	Σa_{cl}	e_{ijklmn}
M1	√	√	√	√	√	√				√	√		√
M2	√	√	√	√	√	√				√	√	√	√
M3	√	√	√	√	√	√	√			√	√		√
M4	√	√	√	√	√	√	√			√	√	√	√
M5	√	√	√	√	√	√		√		√	√		√
M6	√	√	√	√	√	√		√		√	√	√	√
M7	√	√	√	√	√	√			√	√	√		√

[a] Placement Score, or PS (as in Sartori and Mantovani, 2010); [b] μ=overall mean; YB_i*C_j=year-battle*category of weight, 558 levels; HY_k=herd-year, 3,667 levels; AC_l=class of ages, 7 levels; b_j*$W_{m:j}$=individual weight, as covariate by category of weight; d_o*$F_{m:o}$= inbreeding coefficient as covariate by breed; Fc_n=class of inbreeding; d_o*$AR_{m:o}$= average relatedness coefficient as covariate by breed; [c] p_m=permanent environment, a_n=direct additive genetics, a_{Cl}= associative genetic component (i.e., IGE), and e_{ijklmn}=residual term.

According to Sartori and Mantovani (*submitted*), genetic parameters estimated were: i) the overall heritability (h^2), computed either dividing $\sigma^2_{a_D}$ by the sum of all other variance components (i.e., when direct additive variance was the only genetic estimate), or summing both direct and associative heritabilities ($h^2 = h^2_D + h^2_C$), ii) standard errors (SE h^2) for heritability estimates, and iii) repeatability. Direct heritability was assessed dividing $\sigma^2_{a_D}$ by the other variance components too, whereas associative heritability due to conspecifics (i.e., IGEs) was computed as the ratio between $\sigma^2_{a_C}$ and the other variances. SE h^2 were assessed as in Falconer (1989):

$$SE_{\hat{h}^2} = 4\sqrt{\frac{2(1-t)^2[1+(k-1)t]^2}{k(k-1)(s-1)}}$$

where t is the intraclass correlation estimated by (h^2/4) for paternal half-sibs, k is the average number of offspring per sire, and s is the number of sires in the pedigree file. Repeatability accounted for all estimated variance components at numerator and all components including the residual at denominator (Lessels and Boag, 1987).

In order to avoid an overcrowding of analyses accounting for the same parameters but assessed thorough different ways, all the following investigations just referred to the EBVs for fighting ability quantified via classical quantitative analysis (i.e., M1).

ii) Genetic trend of EBVs for fighting ability

A genetic trend for fighting ability was assessed by a linear regression (Standard and Regression procedures, SAS Institute, 2004) on the yearly average EBVs of participants born in the decade 1997-2006. Such range of data has been chosen aiming both to depict a portray of variation in EBVs within a determined time interval (i.e., a decade), as well as to take into account a reasonable amount of data (n=63±45 and n=627±210 for ABP and AC, respectively). Expressed in matrix notation, the regression model was the following:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{e}, \tag{3}$$

where **y** is an N×1 vector of observations, i.e., the average EBV of fighting ability by year, **β** is the vector of fixed effects, here embodied by the birth year, and **e** the vector of residuals. Moreover, **X** is the design matrix of regressors relating the observations to **β**.

EBVs of fighting ability arose from the genetic evaluation of the trait on the classical quantitative model not accounting for either F or AR as fixed factors (i.e., M1, see below) performed via EM-REML analysis as previously described.

iii) Kinship coefficients variations over time

Genetic trends of F and AR estimates were computed both for ABP and AC on the yearly average coefficients belonging to individuals born in the last two decades (i.e., from 1990 to

2009) and included in the herd book. Linear regression coefficients for traits variation over year were obtained applying a regression analysis (Regression procedures; SAS Institute, 2004) as (2). The average number of animals by birth year retained for the analyses was 978±307 among ABP cattle and 4398±1266 among AC, for a total of 19,554 ABP and 87,967 AC individuals.

iv) Estimation of inbreeding effect on fighting ability in cows grouped by founder

In order to appreciate how the EBVs for fighting ability vary in relation to the increase in homozigosity (quantified by the two parameters F and AR), the effects of kinship on fighting ability were investigated through a linear regression analysis (Regression procedure, SAS Institute, 2004). The matrix notation for the two linear models (i.e., either accounting for F or AR) was still as (2), but considering the average EBV of participants grouped by founders as trait, and the mean kinship coefficient (i.e., either F or AR) as covariated fixed effect. Founders were defined as individuals without any genetic relationships with other individuals in the pedigree other than their heirs (Gulisija et al., 2006), and thus assumed as lacking in inbreeding. The significance of the incidence of the related founder on individual kinship was previously assessed via linear model analysis including either AR or F as trait and both individual breed (i.e., ABP or AC) and birth year, as well as the identity of the founder, as fixed factors (*data not shown*). Founders of fighting cattle were attained using a recursive procedure on the pedigree of fighting cows (i.e., 17,724 records) and detecting the father lines of participants. Furthermore, only lineages with at least 10 fighting cows as offspring were retained for the analysis. Thus, resulting dataset consisted in a whole of 33 dynasties, most of them including both ABP and AC individuals (i.e., due to some crosses among breeds over generations). Lineages accounted for a total of 6,087 participants (184±235 heir fighting cows per line, with a minimum of 10 and a maximum of 1,141).

RESULTS

Mean inbreeding values for the participants of battles divided per breed (i.e., Aosta Black Pied or Aosta Chestnut cattle) are reported in Table 2, as well as the amount of animals in each inbreeding class, assessed both among participants and within the two populations by sex. Population data considered only the animals born in the last two decades of the 20th century (i.e., 1990-2009), as reference dataset also for the further analyses on populations. This sub dataset was chosen instead of the complete pedigree in order to avoid any matter about asymmetries in pedigree depth (Gutierrez and Goyache, 2005) among the two ABP and AC cattle pedigree. Thus, average values of kinship parameters estimated on fighting cattle (n=791 and n=7,468 in ABP and AC, respectively) showed a significant difference among the two population as preliminary analysis suggested (GLM procedure, SAS Institute, 2004; *data not shown*). Thus, the average inbreeding coefficient among ABP fighting cattle was 2.73±1.32%, whereas average F of AC participants was 0.81±1.68. AR estimates followed the same tendency, with values of 0.85±0.73 for ABP and 0.40±0.29 for AC fighters. Results agreed with literature, where a greater average heterozigosity within population for ABP has been already attested (Del Bo et al., 2001). Mean inbreeding coefficients of dams assessed in the whole population resembled fighters parameters estimates, as well as sires values did (see Table 3). Population structure by classes of inbreeding revealed that about 80% of individuals in ABP cattle, and more than 90% in AC exhibit a level of inbreeding inferior to 3.125%, but greater than zero. Fighting cows data and pedigree information show a similar structure in the distribution of inbreeding among classes, probably due to the fact that the two pedigrees account for same-aged individuals (i.e., average birth years of fighting cows: 1999±3 in ABP and 2000±3 in AC; average birth years within population pedigree: 1999±5 in ABP and 2001±5 in AC). Moreover, the distribution in classes of F revealed that females exhibit a general lower level of inbreeding in ABP (F<3.125% in 80.85% of ABP females vs. 77.22% in males), whereas levels are similar in AC (F<3.125% in 80.85% of ABP females vs. 77.22% in males). In addition, all ABP males are inbred (i.e., lack of F=0), whereas in AC non inbred females are about 2.5% more than males. Both of breeds are however lacking in very highly inbred individuals, among females as well as among males (F>25% in 10% of ABP and in 11% of AC individuals). Concerning average relatedness, coefficient estimates among fighting cattle and both sexes in the whole population resulted as similar (see Table 4).

Due to the different genetic meaning of the two parameters, the correlation among individual F and AR computed on both breeds and types of dataset (i.e., fighting cows or whole population) revealed scarce or not significant (r<0.20; Correlation procedure, SAS Institute, 2004). About genetic estimates of fighting ability and the incidence of inbreeding on such behavioural trait, the results of the performed analyses are reported in the following paragraphs.

TABLE 4. Percentage of animals in each inbreeding class and average kinship parameters (mean ± standard deviation). Kinship parameters accounted are inbreeding (F) and average relatedness (AR). Fighting cows (a) and the whole population by sex (b, c) are accounted into the table. Information are separately referred to Aosta Black Pied (ABP) and Aosta Chestnut (AC) cattle. Population data concerned animals born in years 1990-2009. Pedigree update: August, 2010.

Classes of inbreeding, (F, %)	Fighting cows (in %)		Population (in %)			
	ABP	AC	ABP		AC	
			Females	Males	Females	Males
F=0	0.00	2.30	0.02	0	4.25	1.76
0<F<3.125	80.78	94.00	80.83	77.22	92.61	95.66
3.125<F<6.25	17.07	1.71	16.27	19.25	1.39	1.18
6.25<F<12.5	1.64	1.18	1.98	3.00	1.04	0.86
12.5<F<18.75	0.51	0.63	0.76	0.51	0.58	0.44
18.75<F<25	0.00	0.12	0	0	0.08	0.05
F>25	0.00	0.05	0.14	0.01	0.04	0.05
Average F (%)	*2.73 ± 1.32*	*0.81 ± 1.68*	*2.74 ± 1.78*	*2.86 ± 1.52*	*0.77 ± 1.56*	*0.84 ± 1.39*
Average AR (%)	*0.85 ± 0.73*	*0.40 ± 0.29*	*1.03 ± 0.77*	*1.00 ± 0.86*	*0.34 ± 0.31*	*0.39 ± 0.29*
No. individuals	791	7,468	12,697	6,857	48,037	39,930

i) Genetic evaluation for fighting ability accounting for F or not

An overview of the genetic analyses carried out on fighting cows data is provided in Table 5. Here, models are grouped by type of analysis, i.e., either classical quantitative or including indirect genetic effects (i.e., associative component). Considerations about the effect of the introduction of kinship components within the genetic model have been separately carried out for the two types of model; an investigation on the different ways to perform a genetic analysis on social traits (i.e., if accounting for IGEs or not) has been already achieved in a previous study (Sartori and Mantovani, *submitted*). In both types of models, the inbreeding coefficient was introduced as covariate by breed (d_o*$F_{m:o}$, M3 and M4), as well as class (Fc_n, M5 and M6). The average relatedness coefficient has been only considered as covariate by breed (d_o*$AR_{m:o,}$ M7) into a classical quantitative model because the most part of considerations concerning F are also suitable for AR. Moreover, eventual classes of AR should have been different from inbreeding classes, due to the diverse variability of the two coefficients. Previous studies carried on fighting cows have already evaluated what fixed factors (apart kinship coefficients) could provide the best fits for fighting ability genetic estimations (i.e., Sartori and Mantovani, 2010). Preliminary ANOVAs on models including kinship revealed that also F and AR have a significant effect on phenotype, both when they are considered as class categories or as covariates (d_o*$F_{m:o}$: *F*=9.61, *P*<0.001; Fc_n: *F*=4.53, *P*<0.001; d_o*$AR_{m:o}$: *F*=7.76, *P*<0.001). Comparing the coefficients of determination (R^2), all models (i.e., including kinship or not) exhibited similar estimates, with a value of about 45.5% On the other hand, kinship components did not produce relevant changes in genetic parameters estimates, both when they were included into classical quantitative models as well as into models with IGEs. The introduction of F or AR as covariate trigged a small shift of additive genetic variance into the permanent environment, leading to a little reduction in heritability (i.e., from 8.3±3.6% to 8.0±3.6%), slightly greater with F. After classical quantitative analyses, heritability estimates were similar (M1, M3, and M7) and consistent with previous estimations (h^2=7.8±4.2%; Sartori and Mantovani, 2010; h^2=7.6±4.3%; Sartori and Mantovani, *submitted*). On the other hand, the introduction of F as class (Fc; M5) led to a shift of the permanent environmental variance into the additive genetic and residual components, producing an increase in heritability (h^2=10±3.7%). Accounting for social effects (i.e., IGEs; M2, M4 and M6), heritability estimates were the same among different models, with a level of 12±3.7% in all cases, included the model with Fc. The previous evaluation of fighting ability through an analogous IGEs model (Sartori and Mantovani, *submitted*) reported a slightly lower value (9.0±4.3%). Concerning repeatability estimates, they almost did not vary with kinship, as well as, accordingly with literature, they attested around 21% in classical quantitative models and 13% in IGEs ones, with slightly lower values in both models with Fc. Model comparison via AIC estimates (Akaike, 1974), confirmed that models fit better when associative components are accounted (i.e., lower values produce better fits; see Table 5 and Sartori and Mantovani, *submitted*). Moreover, comparisons within type of model revealed that including F as class always offered better fits. In addition, the introduction of F provided a better fitting as respect to AR (M3 vs. M7; AIC=111,046.05 and 111,048.26 for F and AR, respectively). However, fitting differences were small, and reasonably not so relevant. As a matter of fact, Pearson and Spearman correlations (Correlation procedure, SAS, 2004) carried on EBVs by type of model always reached levels of 99.9% with a great statistical significance (P<0.001; data not shown in table). EBVs for fighting ability achieved with M1 where thus retained for the following analyses. In addition to what has been explained above (see materials and methods), such estimates were preferred to IGEs results because they don't require to be computed as sum of two components (i.e., direct and associative ones) but they just arise from REML analyses.

TABLE 5. Model fitting parameters, variance components and parameters estimates in different models. The average number of competitors per fighting cows is n = 1.886.

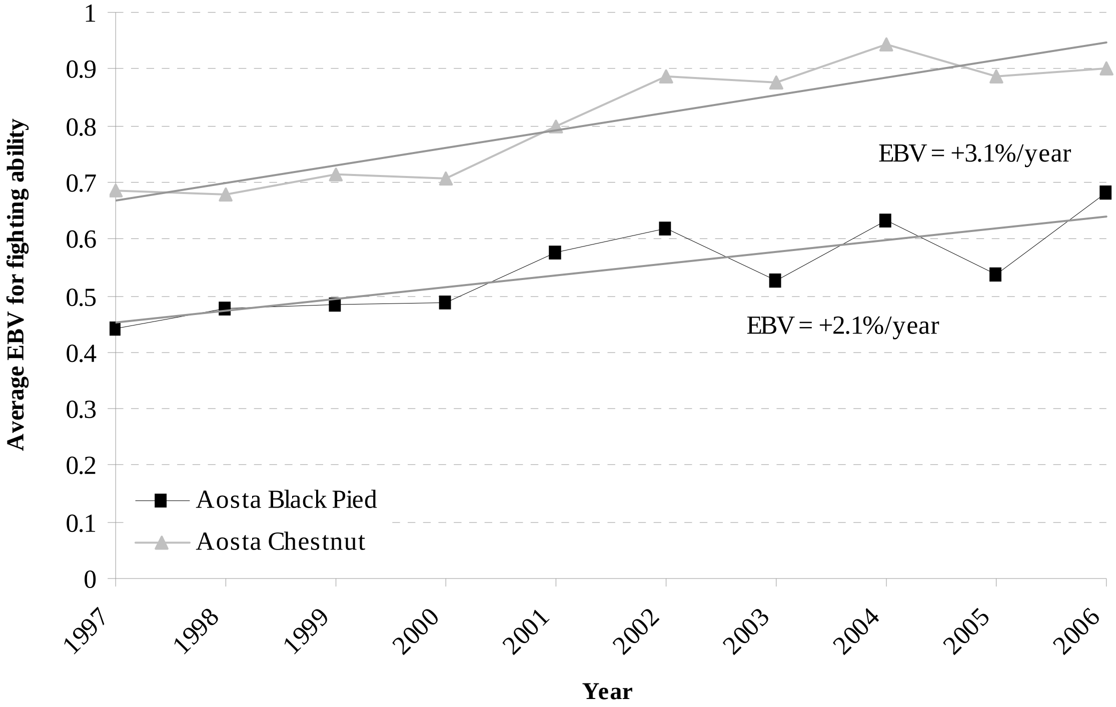
Model	Fitting	Variance Components [b]				Genetic parameters [c]				
	AIC [a]	σ^2_p	$\sigma^2_{a_D}$	$\sigma^2_{a_C}$	σ^2_e	h^2	h^2_D	h^2_C	SE h^2	rep.
				Classical quantitative models						
M1 [d]	111,057.37	1.025	0.632		5.973	0.083	0.083		0.036	0.217
M3 [e]	111,046.05	1.042	0.608		5.973	0.080	0.080		0.036	0.216
M5 [f]	108,452.07	0.844	0.761		6.024	0.100	0.100		0.037	0.210
M7 [g]	111,048.26	1.041	0.610		5.972	0.080	0.080		0.036	0.217
				Models including indirect genetic effects						
M2 [d]	89,123.07	0.019	0.015	0.178	1.401	0.120	0.009	0.110	0.037	0.131
M4 [e]	89,123.31	0.019	0.015	0.178	1.401	0.120	0.010	0.110	0.037	0.131
M6 [f]	87,318.22	0.011	0.016	0.174	1.411	0.120	0.010	0.108	0.037	0.125

[a] Akaike Information Criterion (AIC; Akaike, 1974); [b] σ^2_p = permanent environmental variance (i.e., direct component), $\sigma^2_{a_D}$ = direct additive genetic variance, $\sigma^2_{a_C}$ = associative additive genetic variance (adjusted by the number of competitors); σ^2_e = residual variance; [c] h^2 = overall heritability; h^2_D = direct heritability; h^2_C = associative heritability; SE h^2 = standard error of heritability (Falconer, 1989); rep. = repeatability; [d] Models not accounting for kinship within the fixed factors; [e] Models including the inbreeding F as covariate by breed (i.e., $d_o{*}F_{m:o}$); [f] Models including the inbreeding F as class (i.e., Fc_n); [g] Models including the average relatedness coefficient AR as covariate by breed (i.e., $d_o{*}AR_{m:o}$).

ii) Genetic trend of EBVs for fighting ability

A genetic trend for fighting ability was appraised studying the variations of EBVs for the trait over years. The time interval 1997-2006 was chosen in order to retain a defined period (i.e., a decade), and to select a consistent number of data within years and as respect to the whole dataset of participants (n=633 ABP cows, 80% of the total fighting cattle; 6,272 AC cows, 84% of the total). Thus, regression analyses (Standard and Regression procedures; SAS Institute, 2004) permitted to estimate the incidence of the time on fighting ability as linear regression coefficient (b). Such coefficient attested a positive trend of average annual EBVs both in ABP and in AC. The rate was greater in AC (b=+3.1%/year; *P*<0.001) in comparison with ABP cows (b=+2.1%/year; *P*<0.01), resembling the different fame of the two varieties in the traditional competitions. As a matter of fact, a greater competitiveness, as well as overall better results achieved in tournaments, are commonly attributable to AC breed, generally preferred by farmers for competitions. Hence, average EBVs for fighting ability in participants reveal as greater in AC cows, confirming evaluations that farmers have been assessed just empirically till now (mean EBV in ABP=0.52±0.31, and in AC=0.78±0.38; n=8,259 participants).

Figure 1. Annual trend of estimated breeding values (EBVs) for fighting ability estimated on individual data of fighting cows born in the decade 1997-2006. Number of participants considered: 6,905 cows. Average number of participants per year of born: Aosta Black Pied, 63.3±44.9; Aosta Chestnut, 627.2±210.2.



iii) Kinship coefficients variations over time

Average values of kinship coefficients have been already provided above (see the first lines of materials and methods). The trends in inbreeding and average relatedness for the two breeds are shown in Figure 2 and Figure 3, respectively. As graphs suggest, the increase in kinship parameters was linear for F as well as for AR both in Aosta Black Pied and Aosta Chestnut breeds. In the course of the time, mean inbreeding of ABP population shifted from 1.75±1.28% (i.e., in 1990), to 2.96±0.80% (i.e., in 2009), whereas F values in AC varied from 0.21±1.28% to 1.01±1.02%. Linear regression analyses (Regression procedures; SAS Institute, 2004) carried on year coefficients indicated an overall increase in inbreeding levels within both AB and AC populations, revealing as faster in ABP (b = +0.06%/year; $P<0.001$) than in AC (b = +0.03%/year; $P<0.001$), according to the smaller population size of ABP.

Figure 2. Inbreeding trends in Aosta Black Pied (ABP) and Aosta Chestnut (AC) cattle. Average inbreeding coefficients were computed on the whole population data accounted in the pedigree and referred to individual born in the last two decades (i.e., 1990-2009). A total of 19,554 ABP and 87,967 AC individuals were considered. Average number of animals per year of born: Aosta Black Pied, 977.7±307.3; Aosta Chestnut, 4,398.3±1,265.9.

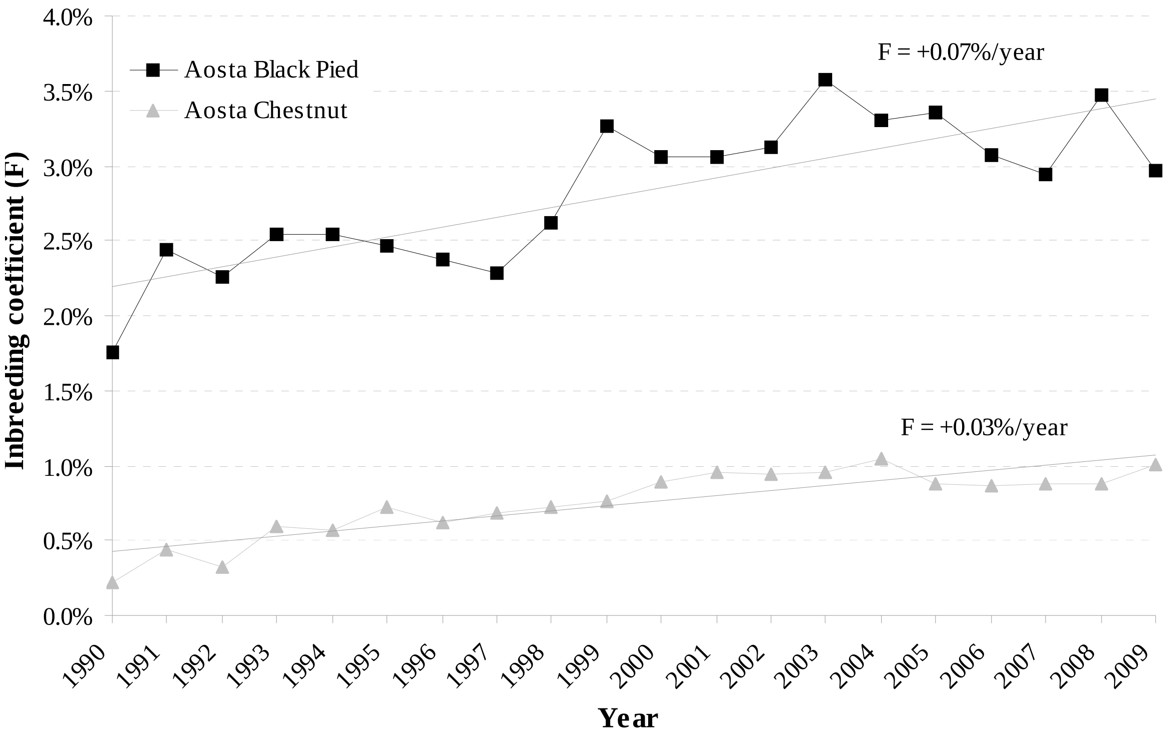
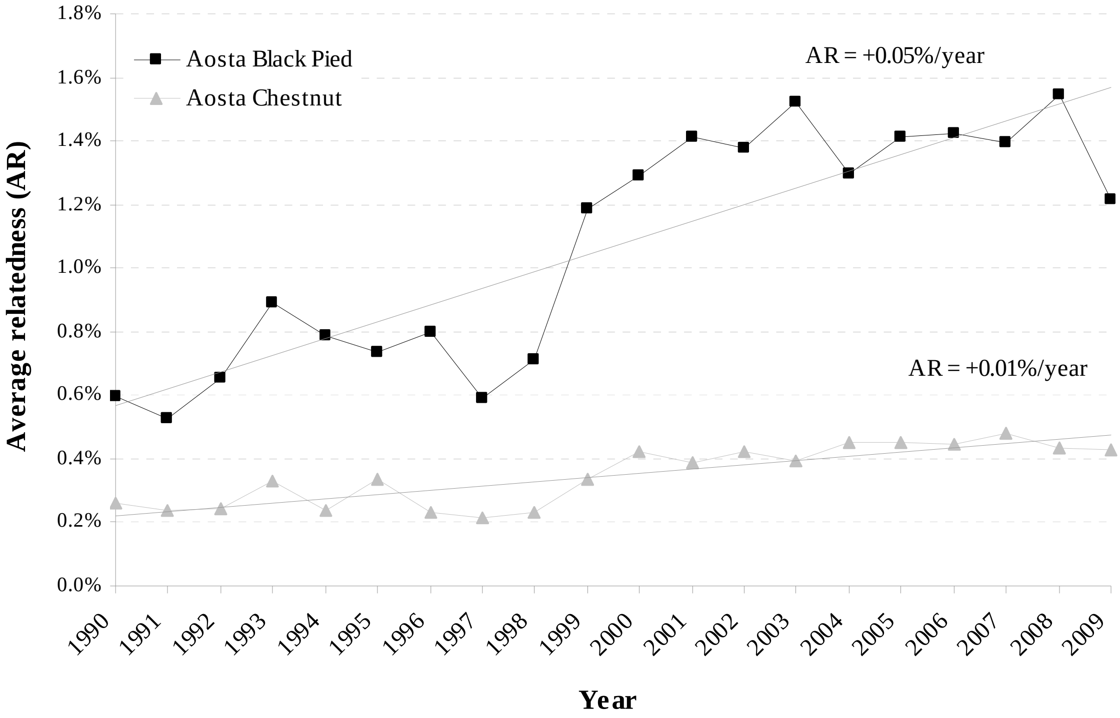
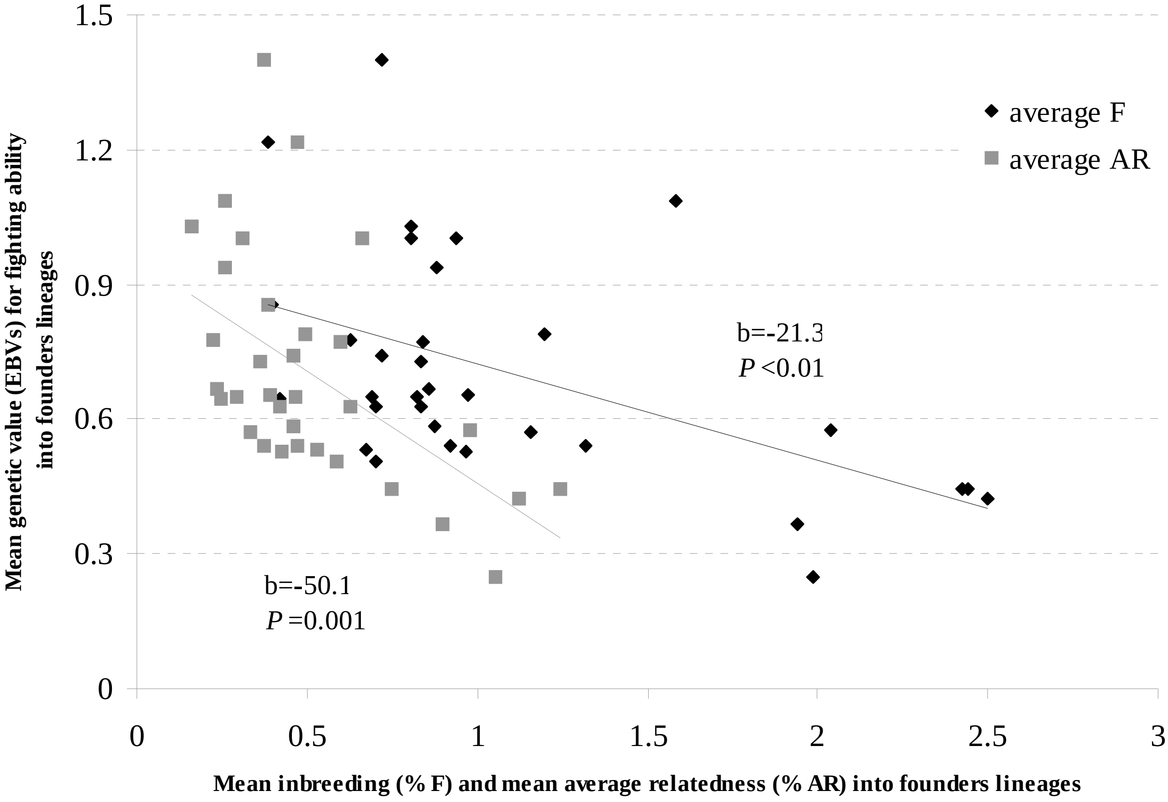


Figure 3. Average relatedness (AR) trends of Aosta Black Pied (ABP) and Aosta Chestnut (AC) populations. Coefficients were computed on the whole pedigree and referred to individual born in years 1990-2009 (i.e., the same of Figure 2).



Such level of inbreeding rate could reveal problematic in ABP, since it is greater than the conventional threshold (F = 0.06%) above whom a breed is considered as endangered (FAO, 2011). The average relatedness changes over time followed the same shape of F, attesting in both breeds an overall increase in both parameters from 1990 to 2009: AR varied from 0.60±1.28% to 1.22±1.02% in ABP, and from 0.26±0.33% to 0.43±0.29% in AC cattle. As well as in inbreeding estimates, mean AR values were greater in ABP as respect to AC population, as well as the rate of increase in coefficients over years (b=+0.05%/year; *P*<0.001 in ABP; b=+0.01%/year; *P*<0.001 in AC). Despite their different meaning, all kinship coefficients are able to sum up some aspects of population dynamics as variation in size, assortative mating or migration that are able to affect the genetic structure of a population.

Figure 4. Genetic values (EBVs) for fighting ability in lineages of fighting cows plotted on the associated Average relatedness (AR) and inbreeding (F) coefficients. All parameters have been assessed as mean individual estimates. The incidence of kinship (F and AR) is expressed by the coefficient of linear regression (b) computed through linear regression analysis. The statistical significance of the regression analysis is also showed in the graph. Data refer to lineages including a minimum of 10 participants to battles (No. lineages = 33; No individuals = 6,087). Due to a partial introgression among lineages over years, data are not segregated by breed (i.e., ABP and AC).



iv) Estimation of inbreeding effect on fighting ability in cows grouped by founder

The last analysis of this work aimed to investigate any possible incidence of kinship on fighting ability. Lineages of cows clustered by founders permitted to obtain groups homogenous in kinship parameters and suitable for catching eventual differences in F and AR among different groups. Linear regression analyses (Regression procedure, SAS Institute, 2004) performed either using F or AR as covariate for fighting ability breeding values, revealed a significant relation among EBVs and kinship for both inbreeding (b= -21.3, *P*<0.01) and average relatedness (b= -50.1, *P*=0.001). Slopes of regression line (b) well quantify the incidence of kinship on fighting ability. Results of both analyses are portrayed in Figure 4, in reason to depict the different incidence of the two kinship parameters on the behavioural trait. As figure describes, fighting ability genetic values resulted negatively affected by both F and AR (i.e., inbreeding depression on the trait). Moreover, increments in AR have resulted to exert a greater negative effect on EBVs, as the greater value of b attests. Separate analyses for the two breeds have not been performed because a segregation of founders by breed is almost not possible. In addition, preliminary mixed model analyses (Mixed procedure, SAS Institute, 2004) carried on the whole dataset of fighting cows and also accounting for founders as category were not able in detect the incidence of kinship in EBVs, (data not shown) probably be due to the data structure. As interesting, the negative relation among combativeness and kinship is well reflected by the famous differences among the two breeds, one smaller in population size, as well as more inbred and worse fighter (i.e., Aosta Black Pied) and the other one with very excellent contestants, as well as less inbred (also a reasonable number of parents is still unknown) and bigger in population dimension. The appraised inbreeding depression on a behavioural trait provides a very interesting subject for reasoning around inbreeding incidence and its negative effect on different traits, as depicted in the following discussion over results.

DISCUSSION

Increments in homozygosis may involve either changes in alleles frequencies (i.e., due to genetic drift or mutation) or redistributions of genotypes (as after assortative mating), both phenomena leading to inbreeding depression (Keller and Waller, 2002). Natural and livestock populations may incur in one or both situations, thus falling in a reductions in fitness and general performances (Charlesworth and Charlesworth, 1987; Kristensen and Sørensen, 2005). Due to the occurrence of strong assortative mating for genetic improvement (i.e., as in many cosmopolitan breeds) or strong reductions in population size (in autochthonous rustic breeds), various cattle populations suffer an inbreeding depression for some productive or functional traits (Burrow, 1993; Smith et al., 1998). Peculiar traits of breeds, and selection over years for enhancing specific attitudes, have led to different situations of inbreeding depression, affecting either milk production and related parameters (i.e., somatic cells, protein and fat percentages) or meat and body conformation's traits when either dairy or beef cattle were involved. Among dairy cattle, reductions in milk yield performances have been already assessed in both Holstein and Jersey breeds form different countries (i.e., Canada: Miglior and Burnside, 1995; USA: Gulisija et al., 2006; Ireland: McParland et al., 2007; South Africa: Maiwashe et al., 2008; Iran: Rokouei et al., 2010). Similarly, the decreases in traits as meat and carcass quality or growth rate have been mostly found out in beef cattle breeds as Alentejana (Carolino and Gama, 2008), Charolais (McParland et al., 2008), Hereford (McParland et al., 2008), Nellore (Santana et al., 2010), and Piedmontese (Fioretti et al., 2002). Moreover, a notable incidence of kinship in fitness performances has been generally assessed for traits as reproduction (i.e., Santana et al., 2010) or survival (i.e., Thompson et al., 2000). Inbreeding depression has been well detected also for some physiological aspects as the adaptation to harsh environments (i.e., Burrow et al., 1998). Concerning behaviour, the effects of kinship on temperament have been already investigated, finding out either cases of significant influence of F on the trait or not (i.e., Burrow et al., 1998; McParland et al., 2007, respectively; see also the introduction paragraph).

Aosta Black Pied and Aosta Chestnut breeds, as well as the other fighting cattle of diverse countries, are unique among cattle due to the importance of behaviour in breed management, even if selection is empirical and not the outcome of planned breeding programmes. Moreover, behaviour is the most difficult trait on which to perform a genetic investigation, mainly due to a strong plasticity and a complex polygenic architecture (Plomin, 1990). Genetic analyses carried on fighting ability in cattle bore out that the overall heritability of the trait resulted not such influenced by inbreeding incidence, as attested by the similar levels of heritability, estimated through different models including kinship (i.e., F or AR; see also Table 5). However, analysis carried out on an higher genetic level (i.e., on lineages of founders; Lacy, 1996) have permitted to disclose the occurrence of a negative incidence of inbreeding also on a behavioural trait. That sheds light on the magnitude of some important evolutionary forces (as inbreeding depression) in affecting genetic variability and consequent phenotypic expression of traits. Hence, it is also interesting to note that, despite a low heritability and the lack of a planned selective opera, a variation on the genetic value for a behavioural trait may be appreciated, and even in the course of a quite small time period (as in decade 1997-2006).

Inbreeding effects on fighting ability and related behaviours have been assessed in the course of the last 15 years in various species. Reductions in aggressiveness and achievements of lower social status have been well recognized in inbred strains of house mice (*Mus musculus*;

Eklund, 1996; Meagher et al., 2000). Similarly, negative outcomes in territorial disputes resulted as more common in inbred shrews (*Sorex araneus*; Välimäki et al., 2007). As well, male Antarctic fur seal (*Arctocephalus gazelle;* Hoffmann et al., 2004) displayed a strong correlation of reproductive success, longevity and competitive ability with internal relatedness (IR, a homozigosity parameter). Inbreeding demonstrated able to reduce aggressiveness also in land-locked salmon (*Salmo salar*; Tiira et al., 2003) and in *Drosophila melanogaster* (Sharp, 1984). Hence, fighting ability was resulted as reduced also in inbred strains of *Daphnia magna* (Salathé and Ebert, 2003). About livestock, relevant effects of inbreeding on competitiveness have been found out in some cases (i.e., chicken, *Gallus gallus*; Cheng et al., 1984) but in other cases investigations did not produced significant effects of estimates (i.e., horses, *Equus caballus*; Dubcovà et al., 2009).

Concerning the two ABP and AC cattle, despite the occurrence of crossbreed practices over time that have reduced genetic distances (see Del Bo et al. 2001 for data on distances), distinct genetic values for fighting ability can be recognized among the two varieties. Actually, ABP suffers for a greater inbreeding depression, that a small population size enhances. On the other hand, the greater competitiveness that AC exhibits, induces farmers to choose almost cows of this breed for traditional competitions, hence boosting the selection for fighting ability. Thus, two opposite forces contemporarily act thus enhancing the differences among breeds. In order to preserve the genetic worth of the two varieties, breeding practices should take into account the specific features and needs of target cattle populations.

At present, selective practices in cattle as well as in other livestock species, have aimed to take into account the effects of inbreeding both on strictly productive traits as well on other important functional traits, as longevity or fertility (i.e., Thompson et al., 2000; Sewalem et al., 2006). Moreover, different kinship parameters, as inbreeding and average relatedness should be taken into account when the incidence of population homozigosity is evaluated. As a matter of fact, low inbreeding levels might underestimate a great level of kinship due to mating within groups of close individuals. At the contrary, average relatedness is able to detect such situations (Goyache et al., 2003). Nowadays, suitable strategies to check inbreeding and to preserve breeds from eventual negative effects are available for livestock (Weigel et al., 2001). For their part, natural populations have already evolved own strategies of inbreeding avoidance (reviewed in Pusey and Wolf, 1996).

Thanks to their peculiar attitude to fight and to the traditional competitions to which they take part, Aosta Black Pied and Aosta Chestnut cattle offer an interesting and actual example on the noticeable impact that inbreeding may play also on a plastic trait as behaviour, also highlighting the role of behaviour in breeding practices and population dynamics.

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General conclusions

Aiming to generate genetic indexes suitable for a possible introduction within the aggregate index of the breed, the genetics of fighting ability has been investigated in Aosta Chestnut and Aosta Black Pied cattle. In order to describe the trait, agonistic behaviour in cattle was firstly investigated with the tools and the methods of optimality approach and the related game theory. Thus, after recognizing that genes play a role in the outcome of phenotypic performances, the genetic components of fighting ability was estimated, starting with a classical quantitative genetic model and then reasoning around the incidence that the inclusion of social partners in terms of indirect genetic effects (IGEs) may have on the variance estimates.

The fighting performances of cattle occurring in the course of the traditional tournaments follow the same dynamics taking place at pasture, where animals fight in couples in order to establish a dominance hierarchy for grazing (see also the Introduction of Chapter 2 for more details). In Chapter 1 of the thesis the conflicts among cows are analysed in terms of behavioural patterns involved and considering how such behaviours develop in the course of the struggles. Hence, the typical dynamics of an escalated contest have been recognized, consisting in a sequence of actions with increasing agonistic intensity that animals perform in order to transmit honest information about their own fighting ability (or resource holding potential, RHP; Parker, 1974). Game theoretical approaches developed over years (see Arnott and Elwood, 2009 for a review, and the Introduction of this thesis, part 1.2) offer a set of theoretical models able to explain what specific agonistic dynamics two contenders are following, and why they behave in the way they do. Moreover, they reason around some factors that may affect a conflict in terms of asymmetries in fighting ability (Maynard Smith and Parker, 1976) or in the light of “winner loser effects” (Hsu et al., 1999). Contests occurring among fighting cattle seem to follow the rules of a “cumulative assessment” (Payne, 1998), where both a reciprocal evaluation of fighting ability and an own assessment act in reason to decide if going on in the conflict or retreating. Both asymmetries in fighting ability and winner loser effect play a role, since weight, age and above all prior contests’ experience may affect the shape and the agonistic effort of the contest. In addition, the asymmetries in the genetic value of fighting is crucial in determining the outcome of a fight (for details, see Chapter 1). That is an empirical evidence of the important role the genes play in depicting the behavioural tactics coming out within a specific environment and under the action of the occurring selective forces. Thus, what is transmitted to progeny is the heritable genetic component of the trait (Falconer, 1989). Both optimality theories and quantitative genetic models agree with such aspect; hence, if optimality and game theories permit to reason around what could influence the expression of a trait, quantitative approaches offer a tool for estimating the magnitude of the genetic components involved. In Chapter 2 and Chapter 3 such genetic evaluation of fighting ability has been realised. In Chapter 2 fighting ability was evaluated through a classical quantitative genetic model; a genetic investigation of behaviour is typically difficult (Plomin, 1990), and more difficulties are created by the generally low heritability of the trait (Mosseau and Roff, 1987). Traditional agonistic performances in cattle offer a wide dataset (i.e., about 16,000 records were used) suitable for a quantitative investigation of a behavioural trait. Moreover, a phenotype as the Placement Score (PS; Sartori and Mantovani, 2010), as well as independent variables as tournament, herd, weight, age and repeated individual measurements have permitted to build up a genetic model able to estimate the additive genetic variance of cows’ fighting ability (Chapter 2). Thus, a heritability of 0.08 has been estimated, that is low but consistent with literature (Mosseau and Roff, 1987; Stirling et al., 2002). It is interesting to point out that both game theoretical models (Chapter 1) and quantitative genetic approaches (Chapter 2) call the same factors (i.e., age, weight etc.) as influencing the phenotype. The evaluation of fighting ability has been then expanded in Chapter 3, that underlines the importance of conspecifics as “heritable environment” (Moore et al., 2002) through models able to account for indirect genetic effects. These models have revealed a better fitting than the classical quantitative models. As a matter of fact, the phenotypic expression of own fighting ability mainly depends by the asymmetries in fighting ability of the interacting conspecifics, as the game theoretical approaches highlighted. Already in 1979, before the advent of interacting phenotypes and IGEs, West-Eberhard observed that “Conspecifics rivals are an environmental contingency that can itself evolve” (West-Eberhard, 1979).

When an evaluation of genetic components of a trait is carried out, it is important to consider the overall setting in which such trait subsists. In addition to selection, other main forces occur in addressing the phenotypic expression of a trait and the genetic architecture that underlies it. Above them, inbreeding may be of major magnitude force. When mating among relatives is common, as in animal breeding practices or when populations suffer for a reduced population size, inbreeding can increase significantly and inbreeding depression can influence both productive and behavioural traits. As carried out in Chapter 4, the investigation of inbreeding occurrence in Aosta Chestnut and Aosta Black Pied populations shed light on the possible incidence of kinship on a behavioural trait as fighting ability. Literature about inbreeding depression on behaviour is scarce, due to the strong influence that the environment exerts. However, analyses performed on traditional fighting competitions have demonstrated that inbreeding may slightly affect variance components estimates of fighting ability. In addition, fighting behaviour suffers the occurrence of kinship, with lower genetic values when inbreeding level (as well as average relatedness) is greater. Such result suggest that inbreeding may be taken into account when breeding programs are carried out. Analyses included in Chapter 4 shed light on another interesting aspect, i.e., there is a positive trend for fighting ability over time. Just a decade of battles has been enough to notice a general increase in the average breeding values in newborn fighter: despite the lack of a planned breeding program, empirical selection by enthusiastic farmers has been able to drive phenotypes and genes toward a desired direction. That suggests another important consideration, i.e., what a selection for fighting ability may imply for fighting cows. Both Aosta Chestnut and Aosta Black Pied breeds are dual purpose cattle, selected for both milk (mainly) and for meat. A provisional investigation on both genetic values for fighting ability and genetic indexes for muscularity has revealed a lack of relation among the two estimates (Sartori and Mantovani, 2009), but further studies are strongly required (i.e., genetic correlations). Concerning milk, the question is particularly interesting, since that a negative relation among traits such as aggressiveness, dominance, fighting ability, versus others as fertility or milk production is well attested in animals (i.e., Staub and De Beer, 1997; Foerster, et al., 2007). Moreover, a depression in milk production has been noticed both in Italian and Swiss (i.e., Heréns) cattle breeds used for fighting, and an urgency for fertility matters is actual in Switzerland. A breeding program also accounting for fighting ability, as nowadays planned for Aosta Chestnut and Aosta Black Pied cattle, should take into account any possible implication among traits. Thus, a forthcoming study on fighting ability and possible relations with muscularity, milk production and fitness would be necessary in the next future. Moreover, all the traits will be studied under the light of a possible inbreeding depression, allowing a more and more exhaustive and detailed framework on the genetics of productive and functional traits in fighting breeds.

Through the investigation of the dynamics of cattle fights and of the genetic components of fighting ability, this thesis may offer an example of multidisciplinary approach in evaluating a social trait. Literature of quantitative genetics of behaviours, and, most of all, of social behaviour is quite scarce, and traditional fights of cattle may reveal as animal breeding may tell a lot in terms of both genetic improvement and evolutionary issues. Concerning to the management of Aosta Chestnut and Aosta Black Pied cattle, this study reveals that a selection for productive purposes is possible also on a behavioural trait as fighting ability that has not traditionally taken into account by domestication and genetic improvement. The upcoming introduction of fighting ability in the aggregate selection index of the breeds, that will join traditional attitude for meat and milk production, will show us the magnitude of selective forces on a behavioural trait in presence of the "heritable environment" of conspecifics.

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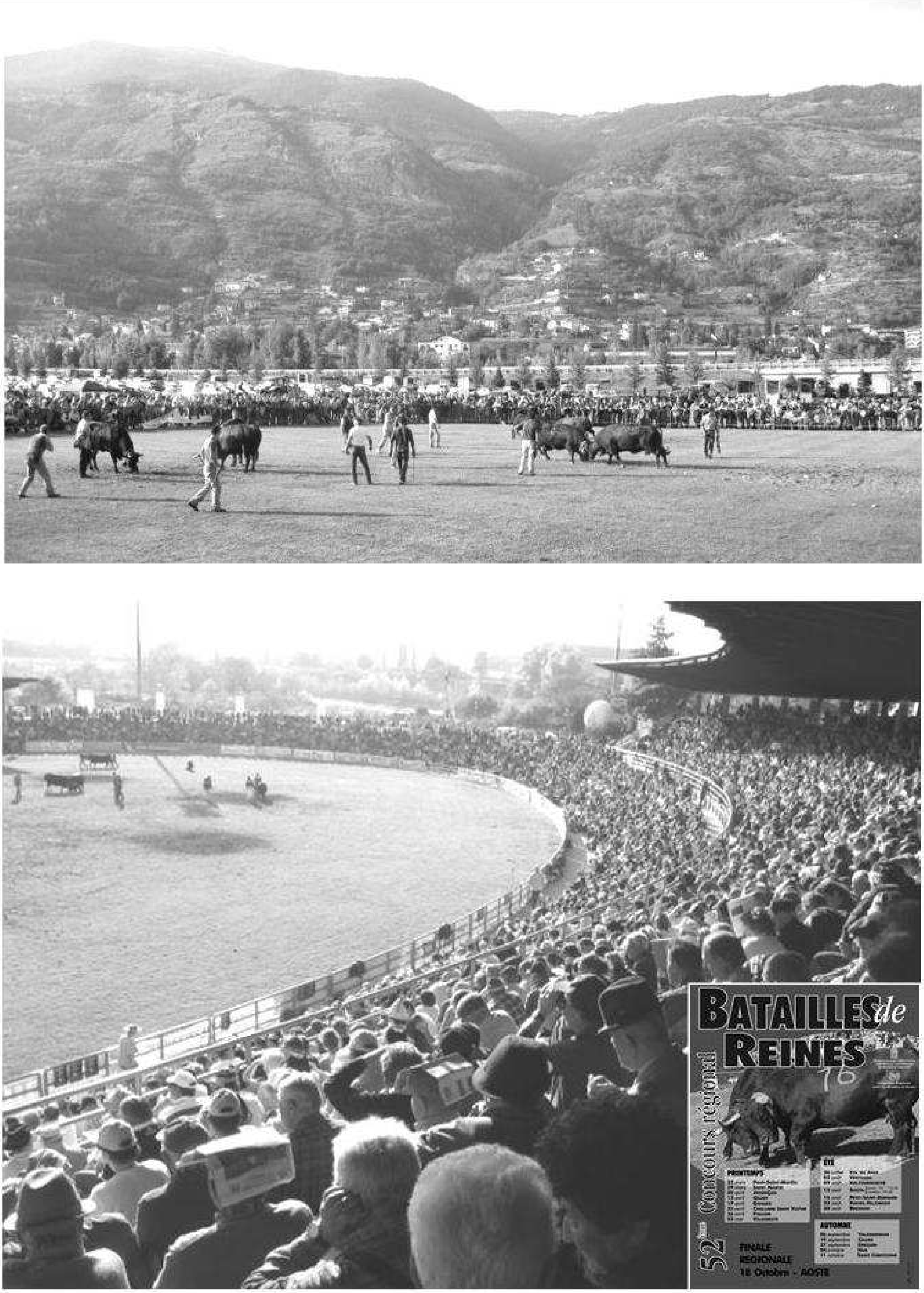
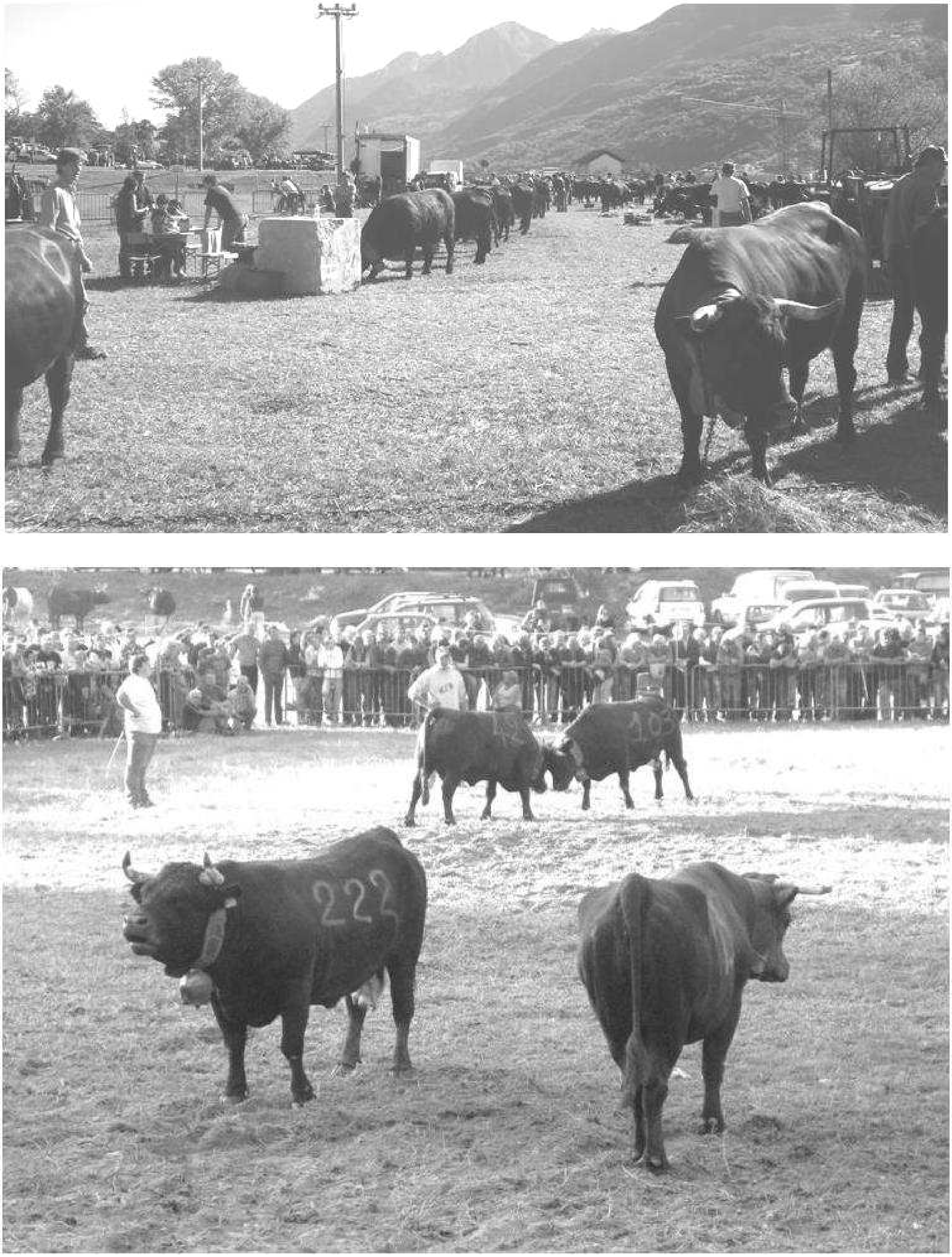
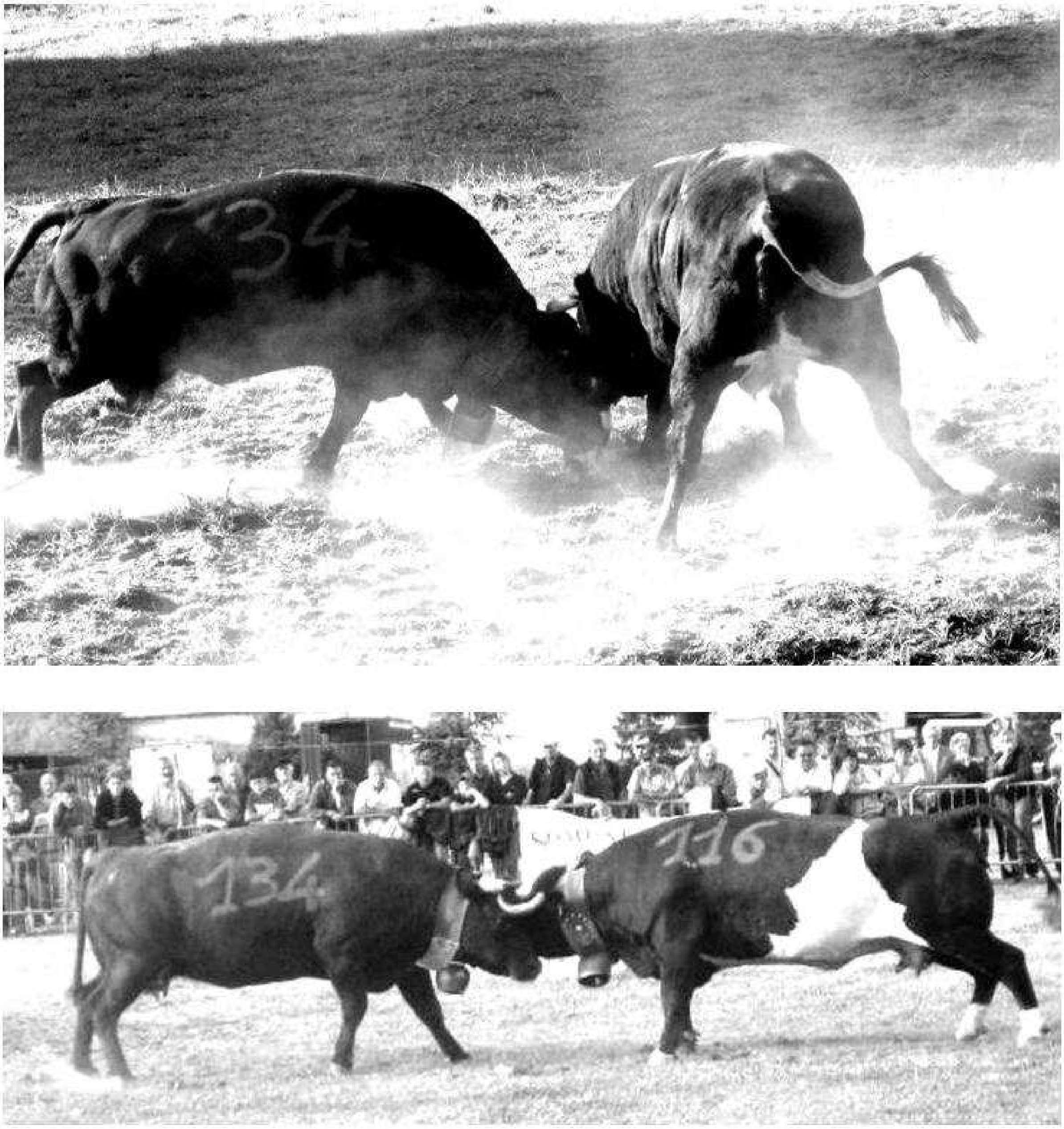
Appendix



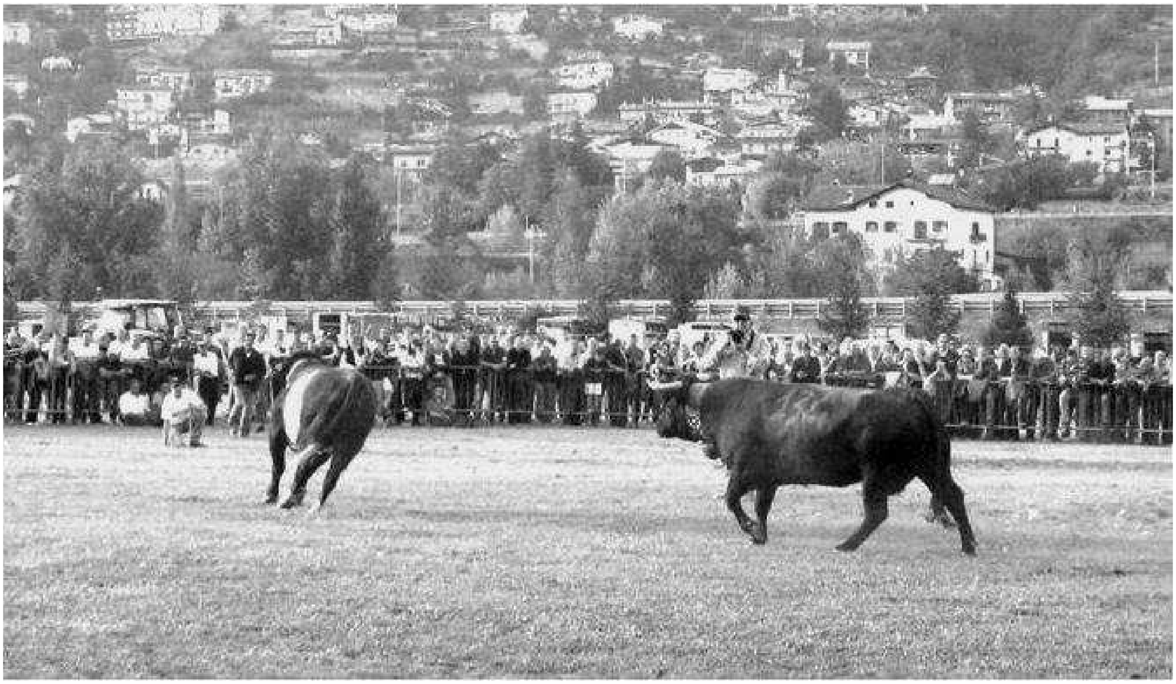
Batailles de Reines in Gressan (AO) (above) and the final tournament in Aosta.



Cows waiting for the fight (above) and cows during the tournament (below): vocalization (cow on the left), visual display (right) and pushing among two contenders (in the background). Arena of Nus (AO).



Vigorous clash among contenders: two Aosta Chestnut cows (above) and an Aosta Chestnut vs. an Aosta Black Pied cattle (below). Arena of Nus (AO).



Victory of one contender and retreat of the loser in the arena of Saint. Christophe (AO) (above); queen of the year 2009, arena of Aosta (below).

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> *“The only baggage you can bring*
> *Is all that you can't leave behind”*
> *(Walk On, U2)*

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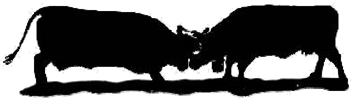

Fighting Cows Project