

## GENETICS OF FEAR AND FEARFULNESS IN DOMESTIC HERBIVORES

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### INTRODUCTION

Animal welfare considerations are now important for farm animal species, and represent a public interest issue for animal production that is additional to concern for product quality or the environment. Animal welfare refers to the capacity for complex thoughts and emotions, and is based on the harmony between the individual and its environment (Dawkins, 1993). Negative emotions such as fear are generally considered to affect animal welfare, and routine management procedures such as shearing, castration, tail docking, dehorning, vaccination, herding and transportation can elicit fear responses. In addition, excessive fear may reduce productivity. For instance, fear reactions affect sexual and maternal behaviours and social dominance ability in cattle and sheep (review Bouissou *et al.*, 2001 ; Fisher and Matthews, 2001). The ability of animals to adapt to their farm environments should be improved by reducing their fear. Genetic variability of behavioural traits is important in domestic herbivores due to the process of domestication. Domestication has changed not only the animals' physical characteristics but also their behaviour such as social grouping tendencies and short flight distances in reaction to humans (Price, 1984). A better knowledge of the genetic factors that influence fear responses could help to increase adaptive abilities that are of economic and ethical significance for farm animals. Evidence that fear responsiveness are heritable has been largely shown in laboratory species (review Ramos and Mormède, 1998), and animal-breeding selection on fear responses could be thus for farm animal welfare as significant as the systems in which the animals are managed. However, little information is available about behavioural genetics in domestic herbivores.

In this review, we attempt to outline the role of genetics in fear responsiveness in herbivores and report the current knowledge about genes and fearfulness. Then, we discuss the limits that may hinder the genetic evaluation and application of fear trait in domestic livestock. We firstly provide an overview of studies in domestic herbivores that emphasises an inter-individual variability and a relative intra-individual consistency in fear responsiveness.

### FEAR AND FEARFULNESS IN HERBIVORES

**Fear reactions as adaptive responses.** Defensive reactions are underlined by negative emotions characterising feelings of insecurity induced by the perception of actual danger, i.e. fear, or potential danger, i.e. anxiety, that threaten the integrity of the individual (Boissy, 1998). From an evolutionary standpoint, defensive reactions promote fitness in wild animals : the life expectancy of an animal is obviously increased if it can react to avoid sources of danger such as predators. In spite of their captivity, domestic herbivores reared in range environments may still experience severe predation by wild animals or dogs. In addition, although natural

selection was relaxed, domestic herbivores still exhibit predator-avoidance strategies similar to wild animals. Avoidance of humans are particularly observed in farm species, though reduced fear of human beings is generally considered to be a major component of domestication (Price, 1984).

**Diversity in the nature of fearful events.** According to Gray (1987), the fear-eliciting nature of an event may be first due to the novelty of the event and to some physical characteristics of its presentation, such as movement, intensity, duration, suddenness or proximity. Fear may be also elicited by specific stimuli, such as height and darkness, in relation to the evolutionary history of the species. In addition, an event can elicit fear by being associated by a previous negative experience. Since the major characteristic of domestic herbivores is their gregariousness, fear may be induced through a variety of social stimuli. First of all, social separation is highly stressful for sheep and cattle. Triggering social signals can also represent particular cases of the previous types of fear-eliciting stimuli. Some social signals are characterised by their novelty, as the novelty of the neonate that affects maternal behaviour in primiparous females. Other social signals, such as odours or alarm calls, can spontaneously elicit fear. Triggering social signals may be also acquired, as in the case of dominance hierarchy.

**Diversity in responses related to fear.** Behavioural patterns related to fear vary greatly depending on the characteristics of the threat. They can be contradictory, since both active and passive strategies are observed in fearful situations: active defence (attack, threat) or active avoidance (flight, hiding, escape) and passive avoidance (immobility) can be viewed as expressing fear (Erhard *et al.*, 1999). Other behavioural patterns can also be considered as fear indicators, including some expressive movements (postures of the head) and some specific alarm calls or odours. Most of these behavioural patterns play an important part in the social communication by serving as signals to alert conspecifics. Fear-eliciting stimuli may also affect the activity in which the animal is engaged. When the level of fear is low, the activity may be enhanced : the administration of weak electric shocks leads to an increase in food intake. In contrast, when fear is intense, the activity can be disturbed or totally inhibited : regular noxious stimuli inhibit social interactions between animals. Finally, conflict between a negative emotion and a positive motivation may result in a compulsive behaviour such as nibbling of a chain.

**Variety of experimental designs.** The experimental situations that have been designed to study the fear in herbivores are various and were at first developed for laboratory species. The open-field test has been extensively used in rodents since it provides at the same time a number of threatening events, such as novelty, absence of shelter or landmarks and bright lighting. Many other tests were devised to assess fear in rodents, such as exposure to a predator or a novel object, confinement, handling, administration of inescapable noxious stimuli, and passive or active avoidance conditioning (review Ramos and Mormède, 1998). This is only more recently that most of these experimental paradigms were used in domestic animals. The open-field design, confinement, exposure to a human, to a natural predator, such as a dog, and to a sudden sound or visual stimulation, and fear-conditioning tests have been widely applied to domestic herbivores (review Boissy, 1998).

**Relative consistency of the fear responses.** Farm livestock show individual differences in fear reactions similar to those found in laboratory animals. Firstly, fear responses can be linked to particular neuroendocrine traits. For example, heifers with strong behavioural reactions to isolation have higher heart rates (Boissy and Le Neindre, 1997). Secondly, individuals can be consistent in their responses across different fearful situations. For example, reactions that express a high level of fear are positively correlated through various tests in heifers (Boissy and Bouissou, 1995) and sheep (Vandenheede and Bouissou, 1993). Thirdly, fear responses refer to stable individual characteristics that show some degree of consistency also over time : measurement of flight reactions towards humans provides a good opportunity to address the issue of fear consistency over time (Burrow, 1997). Given this consistency, it may be possible to assess emotional trait early in life in attempt to predict reactions in further fearful situations.

**The concept of fearfulness.** The numerous intra-individual consistencies suggest that the same emotional process may mediate different fear responses. Such a psychological trait is generally called temperament or fearfulness. In this review, the term fearfulness will be preferred to define a general propensity of an individual to be frightened in the same manner to a variety of potentially fearful events (Boissy, 1998). However, recognising the existence of an emotional trait does not mean that the responses to fearful events can be reduced to simple stereotyped or automatic reactions. By contrast, fearfulness is the result of interactive processes related to genetic background and past environmental influences. In addition, fear reactions are integrated and result from complex interactions between the individual and the external situation. As for the external situation, it is defined not only by the fearful event *per se*, but also by the other physical and social features of the situation that can allow the individual to exert a control on the fearful event.

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**Comparative approaches between breeds.** In studies where domestic herbivores have been reared under the same conditions, large differences in fear responses have nonetheless been observed between breeds. A few studies have used open-field tests to quantify behavioural differences between breeds in sheep (Le Neindre *et al.*, 1993) and cattle (Le Neindre, 1989). From a large variety of tests including surprise effect and novelty, Romanov ewes are more reactive than Ile-de-France ewes (Romeyer and Bouissou, 1992). Similar differences are reported in dairy cattle, with Hérens cows being less reactive to a novel object and to a surprise effect than Brown Swiss cows (Plusquellec and Bouissou, 2001). There are many breed differences in fear reactions to humans in sheep (Le Neindre *et al.*, 1993), beef (Boivin *et al.*, 1994 ; Le Neindre *et al.*, 1995 ; Burrow, 2001) and dairy cattle (Lanier *et al.*, 2000). Fear reactions to human handling are generally higher in Simmentals than in Angus cattle (Gauly *et al.*, 2001) and in Angus than in Herefords (Morris *et al.*, 1994). In cattle, breeds of *Bos indicus* exhibit more fear reactions to handling than breeds of *Bos taurus* (Voisinet *et al.*, 1997).

**Estimating genetic parameters for fear.** A genetic component accounting for part of the inter-individual variability in fear reactions to humans has been found in several domestic herbivores. In a study comparing dam-reared with human-reared goats, Lyons *et al.* (1988) found that among pairs of twins, the goat's rank within the dam-reared group predicted its sibling's rank in the human-reared group. Genetic influences on fear can be clearly shown in

studies of sire effects. In dairy cattle, sire had a significant effect on the reactivity of cows in the milking parlour (Dickson *et al.*, 1970). Similarly, sheep by Romney sires were more reactive when placed in a fearful situation than ewes sired by Suffolk or Columbia rams (Torres-Hernandez and Hohenboken, 1979). In reaction to human, pure-bred Romanov were much more reactive than pure-bred Merinos, whereas Merinos ewes crossbred with Romanov rams were closer to Romanov than to Merinos (Le Neindre *et al.*, 1993). A study was conducted with 1350 lambs from 8 genotypes : Romanov and Lacaune pure-breds, the two crossbreeds (RomxLac and LacxRom) and the crossbreeds of ewes of the four previous genotypes with Berrichon-du-Cher sires (Boissy *et al.*, 1996). In response to social separation, Romanov and the crossbreeds sired with Berrichon rams were more reactive than Lacaune. Nevertheless when a human was close to conspecifics, the Berrichon crossbreeds spent more time than Lacaune near the pen of conspecifics. The genetic analysis revealed that such fear responses were due to direct genetic differences and less to direct maternal influence or to heterosis. In a study involving 560 Limousine daughters from 21 different bulls, the reactions to humans were less variable between cows from the same sire than between cows from different sires (Grignard, 2001).

Estimates of heritability of fear in domestic herbivores are scarce, except for reactions to humans. In dairy cattle, estimates for the heritability of the reactions to humans varied between 0.53 (Dickson *et al.*, 1970) and 0.09 (Wickham, 1979). In beef cattle, a moderate heritability of 0.22 was estimated for reactions to handling (Morris *et al.*, 1994 ; Le Neindre *et al.*, 1995). Variations in estimated heritability may be at least partly explained by methodological differences. For example, when fear of humans is measured by the flight speed upon exit from a handling chute, the heritability was 0.35, whereas it was 0.30 when fear of humans is measured by the agitation score in the chute (Burrow and Corbet, 2000). Furthermore, the estimated heritability can change with the age of the animal. For example, in cattle, the heritability of flight speed was 0.54 at 6 months and only 0.26 at 18 months of age (Burrow *et al.*, 1988).

Most progress on identifying quantitative trait loci for fear has been made with laboratory animals. This approach has been recently extended to the study of fear of humans in farm animals. Schmutz *et al.* (2001) detected seven QTL in calves tested for reactions to humans; one QTL was localised on each of chromosomes 1, 5, 9, 11, 15, and two QTL on chromosome 14. Fisher *et al.* (2001) identified several genetic markers linked with behavioural and physiological responses to humans in Limousin-Jersey crossbred cattle, using an F<sub>2</sub> back-cross design: flight distance was linked to five QTL, plasma cortisol response was linked to one QTL and urine cortisol response to two QTL.

**Possibility of genetic selection to reduce fear.** The heritability estimates for fear responses seem to be sufficiently high to allow further selection on fearfulness in an attempt to improve herbivores management. However, before fearfulness is used as a potential parameter in the genetic improvement, a better knowledge of variances and covariances between the fear trait and productive traits is required for effectively designing new breeding programs. Selection for reducing fear may minimise progress on desirable productivity traits or even select against these traits. For example, selecting silver foxes for a low fear of humans during many

generations resulted in changes in body shape (tail position and ear shape), coat colour pattern, stress and sexual physiology (Belyaev, 1979). However, estimates of relationships between fear and some other behavioural traits are limited in domestic herbivores. In cattle, Grignard (2001) found a negative genetic relationship between the reactivity to humans and maternal behaviour. Likewise in sheep, the ewes that were selected for a low responsiveness to humans exhibited a stronger maternal behaviour than their counterparts that were selected for high reactions to humans (Murphy *et al.*, 1998). Fear of humans is weakly negatively correlated with milk production in dairy cattle (Visscher and Goddard, 1995) and with weight gain (Burrow, 2001) or with days of calving in beef cattle (Fordyce *et al.*, 1996). Consequently, a selection program for reducing fear could be considered since such selection should not have any negative consequences on production traits and, even better, could improve the maternal behaviour.

The development of marker-assisted selection can be useful for psychological traits, such as fearfulness, that are not easy to measure directly on the animal. So far, QTL for fear of humans have been identified using linked markers in cattle (Haley and Visscher, 1998 ; Davis and DeNise, 1998). Such linked markers may be used for marker-assisted selection within sire families once the relationship between the marker and the gene has been determined. Additionally, rapid advances in laboratory animal genomics and the use of candidate gene approaches might permit the identification of specific genes that influence aspects of emotional reactivity in livestock. Knowledge of the precise identity and location of these genes should lessen the risk of selecting against productivity traits when trying to improve fearfulness.

#### **LIMITATIONS TO EFFICIENT GENETIC ANALYSIS OF FEARFULNESS IN HERBIVORES**

**The absence of validation in experimental designs.** Most of the tests used in herbivores for measuring fear have been previously designed for rodents. However, they were generally used in applied ethology without taking into account the biological significance for herbivores. Clearly, there are limitations in extrapolating experimental paradigms between rodents and domestic species, since differences of motivation across species have been identified, such as the social motivation. Therefore, testing animals in inappropriate environments, which is likely to result in abnormal and maladaptive behaviour, can also lead to an inaccurate estimation of fear and may explain the lack of links between studies. A reconsideration of the ecological context of the domestic species has to be conducted in order to elaborate and to validate more realistic conditions of fear stimulation.

**The complexity of fear responses.** Behavioural and physiological reactions to fearful events are only indicators of fear and can not be considered as direct measures of fear. Because of the complexity of the mechanisms underlying fear responses, it is not possible to attribute a given behaviour to any single emotion (Boissy, 1998). For instance, in cattle, the response to a novel object depends also on the experimental designs. When cows voluntarily approach the novel object, the most reactive animals to humans have the strongest tendency to approach it ; by contrast, when the cows are forced to move toward the novel object, just the opposite is true (Murphey *et al.*, 1981). Therefore, a measurement used as an emotional indicator in one

situation can not be extrapolated directly to others, and it is impossible to assess simply the magnitude of fear on the basis of a single "objective and perfect" measurement.

**Genetic and experience interactions.** One aspect that has received less attention, as far as welfare is concerned, is the interaction between the genotype of the animals and the characteristics of their environment. A variety of past environmental factors, by regulating maturation in affective and cognitive processing, may influence the fear responsiveness. Numerous maternal factors are known to influence the emotional reactivity of offspring in laboratory animals. However the effects of prenatal stress on fear responsiveness have received little attention in farm animals (Braastad, 1998). Recently, Roussel *et al.* (2000) showed that transport-induced stress in pregnant dairy goats affects the subsequent fearfulness of their offspring, with the prenatal stressed kids showing more distress in fearful situations. By contrast, the effects of postnatal stimulation on emotional consequences have been largely studied in farm animals. Early handled ungulates are generally less fearful of humans than their non-handled counterparts (review Rushen *et al.*, 1999). Similarly, experiences that occur during adulthood may also influence fear responsiveness. The exposure of adult animals to enriched environments or handling procedures has been shown to decrease subsequent fear reactions, although the reported effects are generally less marked than those induced by the same treatments occurring during infancy. Taken together, early stimulation and later experiences interact throughout life with the genetic background of the individual to continuously modulate its fearfulness. Due to these interactions, some breed differences can change according to particular experiences. For example, whereas Brahmins are generally considered more reactive to humans compared with British genetics, Brahmins become extremely docile if they were handled gently early in life (Torres-Hernandez and Hohenboken, 1979). Therefore, breeding for reducing fear can not be considered independently of the characteristics of the management system. The interactions between the genotype of animals and their environment have to be seen as a critical determinant of animal welfare that could reconcile production with ethical questions.

**Welfare consequences of current breeding programs.** Generally, it is difficult to assess the way the current approaches for genetic selection of domestic herbivores take into account fear and welfare of the animals. Only a few studies suggest that current breeding programs for higher productivity may have adverse consequences for fear, in ways that are not fully understood but that could question the ethical basis of such breeding. The hyper-reactivity in cattle could be the result of an overselection for leanness and rapid growth (Grandin and Deesing, 1998). In addition, selection for a social behaviour trait may affect the fear responsiveness. For instance, Hérens cows, an alpine dairy breed empirically selected for social dominance ability, were less reactive to various fearful events than Brown Swiss cows, a breed not selected for this trait (Plusquellec and Bouissou, 2001). Similarly, ewes selected for superior mothering ability were less frightened by the novelty than unselected ewes (Kilgour and Szantar-Coddington, 1995).

## CONCLUSION

This review highlights the complexity of fear and emphasises the numerous interactions between the individual fearfulness and the characteristics of the environmental stimulation.

Fearfulness is obviously adaptable since it results from the dynamic interaction between genetic background and epigenetic factors, such as early influences and previous experiences. The evidence for genetic variations of fear responsiveness in herbivores is essentially based on breed comparisons. For the few estimates of genetic parameters of fear that are available in domestic herbivores, the genetic correlations and heritability estimates are moderate. The impact of recent molecular genetic tools may help to better characterise the genetic control of fearfulness. It is possible to consider in a future to select domestic herbivores according their fear responsiveness in order to improve their adaptation according to the characteristics of the management systems and thus to facilitate optimal production together with a real respect for the animals' welfare. However, before conducting such a selection, it will be necessary to better define fear responses, since no unique behavioural index can be chosen, and to confirm a relative consistency in the fearfulness. Likewise, it will be also imperative to evaluate the potential consequences of such a selection on other behavioural and production traits. The use of fear responses as new selection criteria in farm animals will be restrained as long as simple and validated methods for detecting individual diversity in fear are not fully developed.

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