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Immunological differences in the global release of the major cat allergen Fel d 1 are influenced by sex and behaviour

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ABSTRACT

The biological function of Fel d 1, the major cat allergen released in the environment, is still unclear despite studies suggesting a putative role in chemical communication. Structural and immunological polymorphisms of Fel d 1 have been described. This study examined how Fel d 1 immunological polymorphism may have a physiological origin by estimating a potential relationship with the sex of cats and cat–human interactions. Samples from bath washes of 21 cats were screened to study antibody binding to Fel d 1 using an ELISA. Personality and Tolerance Handling scores were used to assess the behaviour of the cats.

In the washes, Fel d 1 concentrations were significantly lower in females than in males ($P < 0.05$). Slopes from the ELISA dose–dependent curves varied among the cats: males secreted Fel d 1 variants with higher antibody recognition than females ($P < 0.01$). Females that were aggressive and difficult to handle displayed a diminished slope value, and therefore a weaker Fel d 1 immunoreactivity in global washes, compared to females that were sociable ($P = 0.09$) and easy to handle ($P = 0.07$). This study shows a variable immunological polymorphism of Fel d 1 within a cat population, particularly between males and females, and this polymorphism appears to be related to cat–human interactions.

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Introduction

The major cat allergen, Fel d 1, is an airborne allergen mainly responsible for respiratory diseases in humans such as asthma or allergic rhinitis. The only current treatment is allergen-specific immunotherapy, which is not particularly successful with the cat allergen (Vervloet et al., 2000). Unfortunately, the approach most commonly suggested by physicians is the removal of cats from the domestic environment, even though cat sensitized patients are often reluctant at being separated from their companion animals (Liccardi et al., 2003). As a result, this recommendation is rarely followed and is probably not the solution, particularly since several studies have shown that Fel d 1 is a resistant and ubiquitous molecule in the environment. It is detectable at levels capable of sensitizing and triggering symptoms in susceptible individuals in many public places (Morris, 2010).

Fel d 1 is produced by lachrymal glands, salivary glands, sebaceous glands, is secreted onto the skin and the fur of the animal, and is present in large amounts in the anal sacs (Dabrowski

et al., 1990; Mata et al., 1992; De Andrade et al., 1996). Its production is testosterone dependent (Zielonka et al., 1994) and varies according to sex (Jalil-Colome et al., 1996) and anatomical site of production (Carayol et al., 2000). Fel d 1 belongs to the secretoglobulin family (Klug et al., 2000) and its function is still unknown despite many hypotheses. More specifically, a role in binding and/or transport of lipids and/or steroids has been proposed (Vervloet et al., 1995; Karn, 1998; Pattabiraman et al., 2000), suggesting an involvement of Fel d 1 in chemical communication via a putative function of pheromone binding protein (Carayol et al., 2000).

Fel d 1 is a glycoprotein composed of two 18–20 kDa dimers (Duffort et al., 1991; Kristensen et al., 1997), each composed of two polypeptide chains (Morgenstern et al., 1991). Several Fel d 1 molecular forms have already been described (Morgenstern et al., 1991; Griffith et al., 1992; van Milligen et al., 1992; Kristensen et al., 1997). More precisely, we have recently shown that the major molecular forms of Fel d 1 were different in the anatomical sites of production: in the anal sacs, we have found core fragments of lower molecular size and which are less reactive in ELISA (Bienboire-Frosini et al., 2010).

The physiology of the feline anal sacs is crucial in understanding some critical points about chemical communication (Pageat and Gaultier, 2003). Anal gland secretion is released by frightened cats

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(Bradshaw and Cameron-Beaumont, 2000). In this context, contact with humans may be considered stressful for cats, especially for those reluctant to handling. Scores have been established to estimate tolerance of cats to human handling (McCune, 1995). The friendliness of cats towards people is one of the areas that received attention in the study of individual differences and varies substantially between individual animals (Karsh and Turner, 1988). Traits such as 'friendliness to people', defined as 'willingness to initiate proximity and/or contact' could be reliably ranked to describe the personalities of cats (Feaver et al., 1986).

In the present study, we attempted to assess the role of Fel d 1 in relation to some physiological and behavioural aspects of cats to determine if there was a correlation with Fel d 1 variants. Since we recently demonstrated that a variable content in Fel d 1 variants can affect immunological features of cat extracts (Bienboire-Frosini et al., 2010), we wanted to determine if the immunological properties of Fel d 1 in cat washes could vary with sex or could be influenced by the condition of their environmental interactions and particularly the quality of their relationships with humans (personality and tolerance to handling).

Material and methods

Animals and living conditions

A group of 21 healthy cats (European type, 2–6 year old) were used in this study: 12 non-spayed females in anoestrus and 9 entire males. They received the normal care treatment given at the Research Institute in Semiochemistry and Applied Ethology, Saint-Saturnin-Les-Apt, France. Each activity involving cats was regulated by the French order 2001–464 related to experiments on vertebrate animals.

Cat washing

Cats were washed by immersion as described (Avner et al., 1997), with slight modifications: a wash basin was filled with 5 L of deionised water at 38 °C. Cats were anesthetized with 200 µg/kg of SC medetomidine (Domitor, Pfizer Santé Animale). Each cat was immersed for about 3 min in water in order to collect 95% of the soluble Fel d 1. The entire body, except the surface delimited by the eyes and nose, was soaked and rubbed to ensure adequate penetration of the pelt. Cats were removed from the water and wiped with a clean towel. The washing solution (1 L) was kept and stored at –20 °C until used.

ELISA

The Fel d 1 ELISA kit (INDOOR Biotechnologies) was used according to the manufacturer's instructions to measure Fel d 1 in the washes. Streptavidin-peroxidase conjugate, Tween-20, hydrogen-peroxide solution (30%), bovine serum albumin (BSA), 2,2'-azino-bis(3-ethylbenzothiazole-6-sulfonic acid) diammonium salt (ABTS) were provided by Sigma and 96-well microtitre plates came from VWR International. To study antibody binding, dose-dependent curves (Fig. 1) were established for each cat at the equilibrium by increasing the incubation times, in order to allow for all the variants of Fel d 1 to bind the monoclonal antibodies (mAbs): overnight incubation at 4 °C with the capture antibody and 4 h at room temperature with the tracer antibody. A high slope value reveals strong antibody affinity for Fel d 1 molecules from the various cat samples (Davis, 2005).

Evaluation of cat personality and tolerance to handling

Nineteen cats were scored to have data about the quality of cat–human interactions. Two cats were not scored because data were not available at the time of sampling. To evaluate cat–human interactions we scored each cat's personality and tolerance to handling at the moment of biological sampling (Tables 1 and 2). In this study, socialization, referring to a familiar person, was scored for each cat according to authors' description of the common cat personality types (Feaver et al., 1986; Karsh and Turner, 1988; Casey and Bradshaw, 2008). The score for tolerance to handling was obtained by scoring the cats' reactions to repeated, routine physical examinations and treatments. Scores were given by at least two licensed veterinary behaviourists.

Statistical analysis

The difference in Fel d 1 concentrations between males and females was tested with Wilcoxon–Mann–Whitney two-sample test. Slope values were calculated from the linear regression analysis of the dose-dependent curves using three points

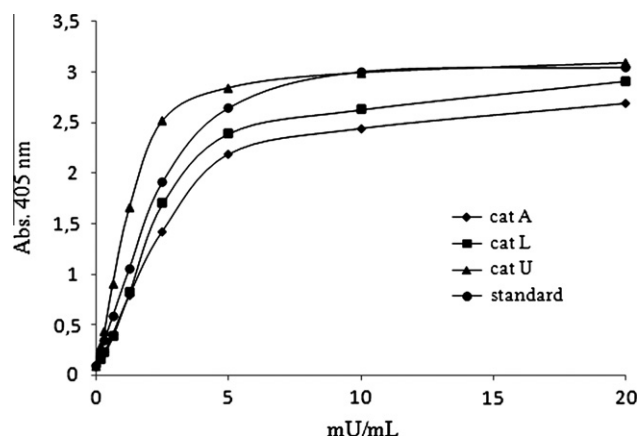


Fig. 1. Dose-dependent curves (0–20 mU/mL) of three representative cats and the standard at equilibrium displaying different slopes in their linear part. The points at 2.5, 1.25, and 0.625 mU/mL were chosen to calculate the slope values of the linear regression analysis ($y = ax + b$), with a coefficient of correlation (r) at least equal to 0.97.

at 2.5, 1.25, and 0.0625 mU/mL and with a correlation coefficient ≥ 0.97 . These slopes, which stand for the antibodies–antigen recognition characteristics (Davis, 2005), were compared between sexes using Wilcoxon–Mann–Whitney two-sample test. In behavioural data, Spearman's ρ was used to measure the covariation between the two variables: behavioural score and slope value, within male and female groups. A Wilcoxon–Mann–Whitney two-sample test was used to compare slope values of Fel d 1 of aggressive and not handled cats with the sociable and easy to handle cats. $P < 0.05$ was considered statistically significant.

Results

Fel d 1 concentrations in washes

Table 3 summarizes the data of Fel d 1 concentrations and slope values obtained from the linear part of the dose-dependent curves established for each cat's wash and the Personality and Tolerance Handling scores. The measured concentrations of Fel d 1 ranged from 19.6 to 1821.5 mU/mL with a mean of 464.4 mU/mL $\pm$ 476.6 and a median value of 225.6 mU/mL. This range shows a rather high heterogeneity of the samples in Fel d 1 concentrations. Cat C, which was one of the youngest cats, was excluded from the following statistical analyses after computation of reduced deviation. The median concentration of Fel d 1 measured in females was significantly lower ($P < 0.05$) to that measured in entire males (Table 4).

Fel d 1 immunological properties and physiological features of cats

We were interested in analyzing potential differences between cat washes and in determining whether variable antibody recognition was present: the lowest slope value was of 0.538 and the highest was of 0.837, with a mean of 0.695 ± 0.094 . The kit standard value 0.704 ± 0.059 matches the value of the mean. These data indicated that each cat wash behaved differently in binding the mAbs, highlighting a complex immunological polymorphism of Fel d 1 within the current cat population.

To understand the biological relevance of these findings, we compared the effect of physiological features on Fel d 1 immunological polymorphism using statistics. Since Fel d 1 production is sex-dependent and under testosterone dependency (Zielonka et al., 1994; Jalil-Colome et al., 1996), we compared the slope values according to the sex of the cats. Despite a large interindividual variability of Fel d 1 immunological properties in both sexes, the slope median was significantly higher ($Z = 2.6617$; $P < 0.01$) for

Table 1
Personality score.

Score	Definition	Description
1	Sociable, confident, easy-going, and trusting (Feaver et al., 1986)	The cat, during the approach, immediately shows all the signs of greeting: tail rise, kneading paws, rubbing the person and drooling
2	Timid, nervous (Feaver et al., 1986), shy and unfriendly (Karsh and Turner, 1988)	The cat, during the approach, moves back to the back of the pen, adopts a flattened posture, with his tail tucked under, lowered head and avoiding eye contact
3	Aggressive (Feaver et al., 1986; Casey and Bradshaw, 2008)	The cat, during the approach, shows signs of threats toward the people; hisses, spits or lashes out at people when approached

Table 2
Tolerance Handling score (adapted from Casey and Bradshaw (2008)).

Score	Definition	Description
1	Easy to handle	The cat is ready and willing for all manipulations
2	Hard to handle	The cat is easy to take but potentially nervous during some manipulations
3	Aggressive during handling	The cat hisses, spites or lashes out at people during the approach and during all types of manipulations

Table 3
Cat features, Fel d 1 concentrations of cat washes, slope values of linear part from dose-dependent curves, and behavioural scores.

Cat	Age (years)	Sex ^a	Fel d 1 concentration (mU/mL)	Slope value	Personality score	Tolerance Handling score
A	4	F	434.7	0.538	3	3
B	3	F	57.9	0.541	3	3
C	2	M	205.5	0.555	2	2
D	3	F	421.4	0.563	3	3
E	4	F	554.8	0.632	1	1
F	3	F	131.6	0.634	2	3
G	4	F	22.7	0.652	3	3
H	5	F	225.6	0.675	3	3
I	4	F	973.7	0.677	2	2
J	4	M	885.5	0.679	1	1
K	4	M	1821.5	0.682	1	1
L	3	M	538.2	0.701	2	1
M	3	F	59.9	0.743	n.d. ^b	n.d.
N	4	F	19.6	0.762	1	1
O	3	F	170.8	0.775	n.d.	n.d.
P	6	M	1157.4	0.787	1	1
Q	4	F	113.4	0.790	1	1
R	3	M	617.3	0.792	3	3
S	6	M	111	0.794	3	3
T	2	M	163.4	0.796	3	3
U	2	M	1066.8	0.837	2	1
Standard	/	/	20	0.704		

^a F, females; M, males.^b n.d., not determined.**Table 4**
Comparison of Fel d 1 concentration and slope values for the sex of the cat (Mann–Whitney–Wilcoxon two-sample test).

Sex	n	Fel d 1 concentration mean $\pm$ s.d. ^a (mU/mL)	Fel d 1 concentration median	Mann–Whitney	Statistical significance
Female	12	265.5 $\pm$ 285.2	151.2	Z = 2.276	P = 0.023
Male	8	795.1 $\pm$ 564.6	751.4		
		Slope value mean $\pm$ s.d.	Slope value median		
Female	12	0.665 $\pm$ 0.089	0.664	Z = 2.662	P = 0.008
Male	8	0.758 $\pm$ 0.061	0.789		

^a s.d., standard deviation.

males (without cat C, excluded from the statistical analysis as previously explained) than for females (Table 4).

Fel d 1 immunological properties and behaviour of cats

We found a correlation in females (Fig. 2) between behavioural scores and slope values both for the Personality score (Spearman's $\rho = 0.617$ and $P < 0.0001$; $\rho^2 = 0.38$; 38%) and Tolerance Handling

score (Spearman's $\rho = 0.605$ and $P < 0.0001$; $\rho^2 = 0.37$; 37%). One female (cat E, slope value 0.632; personality score, 1; Tolerance Handling score, 1) provided questionable results since its behavioural evaluation was variable. We therefore decided to exclude this female from the group and still found a correlation with a slightly increased significance: Personality score (Spearman's $\rho = 0.774$ and $P < 0.0001$; $\rho^2 = 0.6$; 60%) and Tolerance Handling score (Spearman's $\rho = 0.836$ and $P < 0.0001$; $\rho^2 = 0.7$; 70%).

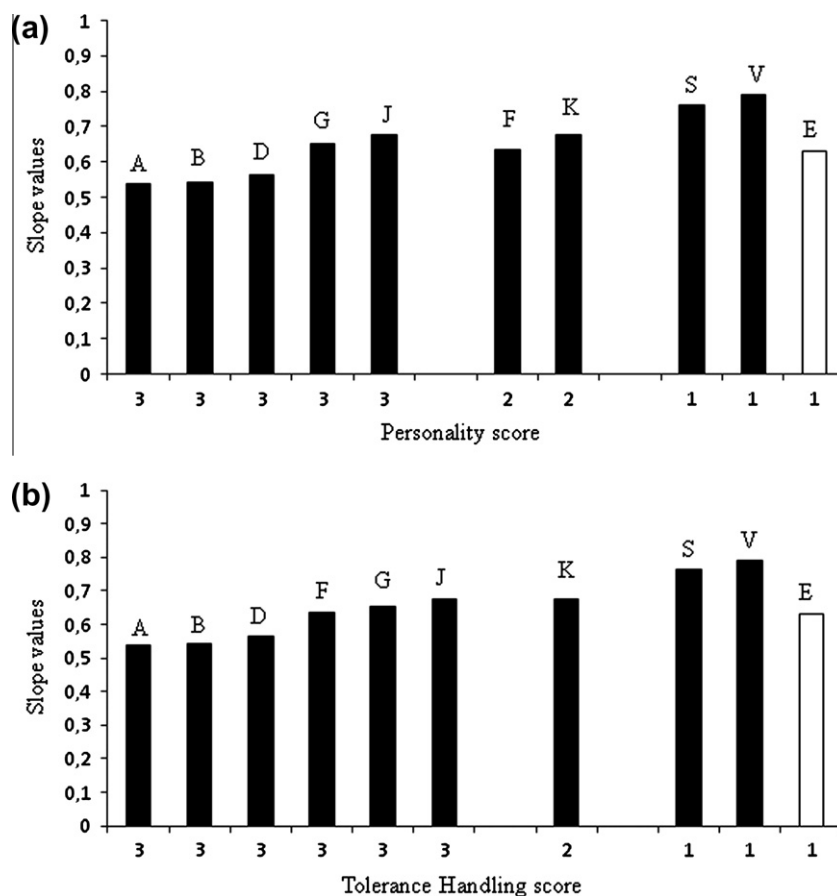


Fig. 2. Distribution of slope values in function of the behavioural scores in females (a: Personality score; b: Tolerance Handling score). Cat E was excluded from the group in the second analysis of correlation since its behavioural evaluation was changeable.

As shown in Table 5, aggressive females (Personality score 3) showed lower slope values than other score categories for females, sociable and shy (Personality scores 1 and 2) ($P = 0.09$). In addition, females aggressive during handling (Tolerance Handling score 3) showed lower slope values than easy or hard to handle females (Tolerance Handling scores 1 and 2) ($P = 0.07$).

Discussion

Fel d 1 polymorphism has been well established over the years (Griffith et al., 1992; van Milligen et al., 1992; Kristensen et al., 1997; Bienboire-Frosini et al., 2010). Here, we were able to correlate in an innovative way variation in Fel d 1 immunological properties with the sex and behaviour of cats, particularly by taking into consideration cat–human interactions. Our study showed that cat washes show dose–dependent curves in ELISA with much lower slopes in females and particularly in females with an aggressive

behaviour. Accordingly, Fel d 1 exhibited a different content in variants among the study cats.

Our findings are in agreement with published studies showing higher amounts of Fel d 1 produced by males (Zielonka et al., 1994; Jalil-Colome et al., 1996) since we also observed a significant difference in Fel d 1 levels between entire males and females. In addition, we noted a definite lack of immunological similarity between Fel d 1 samples in males and females as demonstrated by distinct slope values. These findings cannot be explained by the calibration of the tests since the kit standard is from cat hair and matched perfectly the average value of the group of cats. Rather, it is more likely that the sample contents in Fel d 1 variants differs among male and female cats, leading to these immunological differences.

The cat bathing method to get a wash extract precluded a western blot analysis of Fel d 1 structural polymorphism because of extensive dilution. However, in a recent study using a distinct experimental procedure (Bienboire-Frosini et al., 2010), we were

Table 5
Comparison of slopes values for Personality and Tolerance Handling scores (Mann–Whitney–Wilcoxon two-sample test).

	<i>n</i>	Slope value mean $\pm$ s.d. ^a	Slope value median	Mann–Whitney	Statistical significance
<i>Slope effect Personality score</i>					
Aggressive females	5	0.594 $\pm$ 0.065	0.563	<i>Z</i> = −1.671	<i>P</i> = 0.094
Sociable and shy females	5	0.699 $\pm$ 0.073	0.677		
<i>Slope effect Tolerance Handling score</i>					
Aggressive females	6	0.601 $\pm$ 0.060	0.599	<i>Z</i> = 1.812	<i>P</i> = 0.070
Easy and hard to handle females	4	0.715 $\pm$ 0.073	0.720		

^a s.d., standard deviation.

able to show by mass spectrometry analysis that Fel d 1 could exist as core fragments of low immunoreactivity in the four anatomical zones studied, indicating that these fragments may be spread on the cat's entire body and may therefore be found in the global wash samples. As a result, the presence of these fragments in cat washes could diminish the slope value of any given dose-dependent curve of Fel d 1 (Bienboire-Frosini et al., 2010), as we observed in females in the current study. This variability in slope values among cat samples may introduce discrepancies in the determination of Fel d 1 concentration by ELISA. In this prospect, the observation made in this study and by others (Jalil-Colome et al., 1996) of differences in Fel d 1 production between males and females might eventually be reconsidered.

The current findings show that behaviour may have an impact on Fel d 1 immunological properties with aggressive behaviour in females correlating with low slope values. Since anal sacs are emptied by frightened cats (Bradshaw and Cameron-Beaumont, 2000), it is conceivable that increasing the content in weakly immunoreactive core fragments from anal sacs in cat extracts would result in a decrease of the slope value when a dose-dependent curve is calculated. In addition, when slope values and cat-human interactions were compared, statistical trends indicated that difficulties in handling the female cats may be correlated with a diminished slope value in the Fel d 1 ELISA. We can therefore hypothesize that female cats, qualified as aggressive during their relationships with humans, may have expressed their anal sacs during the washings, thereby releasing core fragments of Fel d 1 in the water extract. Such releases of core fragments would in turn cause altered immunological properties of Fel d 1 samples. Interestingly, we did not found such a correlation in males. This may be related to the difference in sex group size.

Alternatively, the differences in release of core fragments between sexes during cat-human interaction may be linked to female-specific behaviour patterns for cats during social interactions. In several studies (Barry and Crowell-Davis, 1999; Macdonald et al., 2000; Hurd, 2006; Bonanni et al., 2007), the behaviour of female cats has been shown to be more variable than that of male cats. Therefore, all interactions may represent a challenge to which the females may respond in different ways.

It is noteworthy that sensitized human patients report variations of clinical manifestations according to the cat they meet (D. Vervloet, personal communication). Changes in Fel d 1 polymorphism in a cat population as observed in this study are consistent with this observation and provide a molecular basis for further studies. Finally, the correlation between Fel d 1 polymorphism and the cat's emotional state noted in this study supports a role for Fel d 1 in feline chemical communication.

Conclusions

The results showed a correlation between Fel d 1 immunological polymorphism and sex, personality and tolerance to handling in a cat population. Further studies are necessary to confirm these preliminary results. We suggest that this innovative approach, correlating some biochemical aspects of Fel d 1 and cat-human interactions, represents an interesting base to explore the role of Fel d 1 in feline behaviour and the quality of relationships between cats and humans, especially as it relates to humans developing allergies.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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