

# Three statistical methods to analyze breed differences in the behavioral response to a challenging situation in kittens

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## Introduction

In a previous study [1], the behavior of Oriental, Siamese and Abyssinian kittens (OSA-k) was compared with that of Norwegian Forest kittens (NFO-k). Interesting breed differences emerged. In the present study, further statistical analyses were carried out at different levels of complexity to supply a broad set of results. Outcomes were matched together in order to satisfactorily explain behavioral differences observed in the kittens during the response to a challenging situation.

## Materials and methods

Pure breed kittens were supplied by ANFI (Associazione Nazionale Felina Italiana) and SCC (Serenissima Cat Club) cat breeders. 43 OSA-k and 39 NFO-k were exposed weekly to a 12 minute Open Field Test (OFT) from the 4<sup>th</sup> to the 10<sup>th</sup> week of age. The OFT arena was a white rounded box (1,80 m diameter); on the floor there was a cylindrical container as novel object (NO). In each session a randomly chosen kitten was introduced in the arena. After six minutes (First Part of the test - FP), a potentially aversive stimulus (AS) was produced by the sudden opening of the NO and the abrupt exit of a metal spring. The test lasted 6 more minutes (Second part of the test - SP) and the kitten was then removed from the arena.

## Data collection procedures

**Animal identification:** schedule, pedigree name, mother, sex and coat colour were noted for each animal.

**Behavioral data:** all OFT were recorded with a video camera (Sony HandyCam<sup>®</sup>) placed outside the arena. Videos were downloaded on a PC using Microsoft<sup>®</sup> Windows<sup>®</sup> MovieMaker 5.1. Two trained operators registered the duration of behaviors over a total of 720 seconds (360 seconds - FP; 360 seconds - SP), according to predefined categories by focal animal sampling [2].

## Data analysis

All data were analyzed using SAS 9.1 (SAS 2003, USA). The following statistical analyses were carried out:

**Principal Components Analysis (PCA):** data were introduced as mean values across animals to explore association between behaviors, breed, week and FP/SP.

**Analysis of Variance** with repeated measurements (ANOVA); to compare mean durations of each behavior among breeds, FP/SP and weeks (considered as a factor) and their interactions;

**Generalized Random Effect Model**, with two different specifications for the error term:

- **Gamma distribution**, with durations as dependent variable, in order to evaluate behavioural trends along

weeks, defined as a covariate; quadratic term was included to assess the existence of non-linear trends;

- **Binomial distribution**, where the dependent variable was a dummy variable describing the presence or absence of a single behavior; the model assessed changes in the probability of performing a certain behaviour in breeds, FP/SP and their interactions.

## Results and discussion

The PCA showed two components explaining over 90% of the variability in behavioral patterns. The first principal component could describe "anxiety", with "anxious" behaviors like *crouched* and *escape attempts* on one extreme and "relaxed" behaviors, like *walking and exploring floor* on the other; anxious behaviors appeared to be associated with SP in both breeds, suggesting that AS was actually frightening; NFO-k were associated with *escape attempts*, OSA-k with the *crouched* posture. The second principal component seemed to be linked to "interest in exploration" or "curiosity", as there was a regular increase of explorative behaviors through the axis. Behaviors indicative of curiosity were on the same side of the plot of NFO-k during FP and SP.

The hypothesis was that OSA-k would passively face a challenging situation. In NFO-k, both novelty and fear would induce an active strategy. Our suggestions were validated by the further statistical analysis as described above.

ANOVA showed that, during FP, NFO-k explored more than OSA-k ( $p < 0.001$ ), but exploration decreased only in NFO-k after AV ( $p < 0.0001$ ); during first contact with NO, NFO-k spent more time exploring it ( $p = 0.021$ ) than OSA-k; but they increased the latency to enter in contact with it after AS ( $p = 0.011$ ). After AS, NFO-k presented a higher increase in resting postures and spent more time in escape attempts ( $p = 0.034$ ). In general, OSA-k receded more than NFO-k ( $p < 0.0001$ ), but in the latter breed the behavior increased after AV ( $p = 0.003$ ). The Binomial Model showed that in both breeds, for all behaviors and among weeks, the longer time animals spent performing a behavior (results from ANOVA), the higher the number of animals that exhibited it.

Analyzing evolution of behaviors with the Gamma Model, interesting changes appeared with time: in NFO-k emerged a strong variation in exploration levels, that increased during the first weeks, achieved its maximum value during central weeks and then decreased in the last weeks. In OSA-k such a modulation did not appear clearly, denoting less interest for the environment. Conversely, in OSA-k this pattern appeared for the behavior *escape attempts*, whereas in NFO-k this behavior was maintained at the same higher level until the last week.

## Conclusions

Globally examined, our results match well with similar results on temperament obtained in other species [3]. A future

research development would be to use a *Multivariate Regression Model* to analyze behavior as a whole, exploring the covariance structure among several behaviors during a fixed time period and the extent of correlation between this structure and the other explanatory variables.

## References

1. Marchei, P., Diverio, S., Fatjó, J., Ruiz-de-la-Torre J.L., Manteca, X. (2007). Breed differences in behavioural development in kittens. *Proceedings of the 6<sup>th</sup> International Veterinary Behaviour Meeting (Italia, 17-20 June)*.
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