**WHEN ANCESTORS TRANSMIT THEIR STRESS: PRENATAL MATERNAL STRESS TRANSMISSION ACROSS GENERATIONS IN A PRECOCIAL BIRD**

**README FILE**

**Excel sheets description**

Each excel sheet contained informations about individual identity (“F1\_id”, “F2\_id”, etc.), individual treatment (NS = Non Stressed or S = Stressed), individual sex (F = Female or M = Male).

* **“EGGS\_F1” and “EGGS\_F2”**

Sheets named “EGGS\_F1” and “EGGS\_F2” contained data of egg quality (egg weight compounds (“w\_xxxx”)) and yolk hormones (per ng/yolk (“xxxx\_g”) or per yolk (“xxxx\_yolk”)) for both F1 and F2 females.

w\_egg : weigth of the egg (g)

w\_shell : weigth of the shell (g)

w\_yolk : weigth of the yolk (g)

w\_albumen : weigth of the albumen (g)

andro\_yolk : androgens (testosterone + androstenedione) in the yolk (ng/yolk)

andro\_g : androgens (testosterone + androstenedione) in the yolk (ng/g)

proges\_yolk : progesterone in the yolk (ng/yolk)

proges\_g : progesterone in the yolk (ng/g)

* **“WEIGHT\_F2” and “WEIGHT\_F3”**

Sheets named “WEIGHT\_F2” and “WEIGHT\_F3” contained data of F2 and F3 offspring’s weight from hatching to post-hatching day 22.

day : weighing day (hatching (“Hatching”) or post-hatching day 22 (“J22”))

weight : weight of the bird (g)

* **“PCA\_DATA\_F2” and “PCA\_DATA\_F3”**

Sheets named “PCA\_DATA\_F2” and “PCA\_DATA\_F3” contained data we used to run the PCA analysing F2 and F3 offspring’s emotional reactivity. Each sheet included data for variables we recorded during the tonic immobility test (IT\_xxxx), the emergence test (EM\_xxxx) and the novel object test (NO\_xxxx). Empty cells for the emergence test variable signify that the individual did not emerge from the starting box (the test was stopped and variables normally noted in the device phase were not recorded). Empty cells for the novel object test signify that the individual was not tested at all (death, injuries, illness, etc.). During the statistical analysis, all missing data were automatically substituted by the mean value of each variable.

IT\_IND : Number of induction (1 to 5)

IT\_DUREE : Tonic immobility duration (s)

EM\_LTET : Latency of the head to emerge from the starting box (s)

EM\_LATP : Latency of the feet to emerge from the starting box (s)

EM\_LATC : Latency of the whole body to emerge from the starting box (s)

EM\_LCA\_BOX : Latency to emit the first rally call in the starting box (s)

EM\_CA\_BOX : Number of rally calls produced in the starting box

EM\_LCA\_DISPO : Latency to emit the first rally call in the device (s)

EM\_CA\_DISPO : Number of rally calls produced in the device

EM\_OM : Occurrences of median observation behaviour

EM\_OBS : Occurrences of device observations

EM\_EXP : Occurrences of device explorations

EM\_PAC : Occurrences of pacing (stereotypic behaviour)

EM\_PEUR : Occurrences of fear behaviour

EM\_CONF : Occurrences of comfort behaviour

NO\_LAPP : Latency to approach the novel object (s)

NO\_OM : Occurrences of median observation behaviour

NO\_OBS : Occurrences of device observations

NO\_EXP : Occurrences of device explorations

NO\_PAC : Occurrences of pacing (stereotypic behaviour)

NO\_PEUR : Occurrences of fear behaviour

NO\_CONF : Occurrences of comfort behaviour

NO\_OBO : Occurrences of novel object observations

NO\_EXPO : Occurrences of novel object explorations

* **“PCA\_FACTORIAL\_SCORE\_F2” and “PCA\_FACTORIAL\_SCORE\_F3”**

Sheets named “PCA\_FACTORIAL\_SCORE\_F2” and “PCA\_FACTORIAL\_SCORE\_F3” contained the factorial scores we extracted from F2 and F3 PCA and that allowed us to study differences between non stressed and stressed F2 and F3 offspring’s.

PC1 : Factorial scores of the individual for the first component selected

PC2 : Factorial scores of the individual for the second component selected

(PC3) : Factorial scores of the individual for the third component selected

* **“IMMUNO\_H3K27\_F3” and “IMMUNO\_H3K4\_F3”**

Sheets named “IMMUNO\_H3K27\_F3” and “IMMUNO\_H3K4\_F3” contained data of H3K27me3 and H3K4me2 levels in different regions of F3’s brain. Empty cells signify that the levels of H3K27me3 and H3K4me2 can not be measured for this individual/brain region because of some technical issues.

PVN : Level of H3K27me3 or H3K4me2 in the paraventricular nucleus of the hypothalamus (cells/µm²)

Hp : Level of H3K27me3 or H3K4me2 in the hippocampus (cells/µm²)

Ad : Level of H3K27me3 or H3K4me2 in the dorsal amygdala (cells/µm²)

Am : Level of H3K27me3 or H3K4me2 in the median amygdala (cells/µm²)

Ai : Level of H3K27me3 or H3K4me2 in the intermediate amygdala (cells/µm²)

PoA : Level of H3K27me3 or H3K4me2 in the posterior amygdaloid (cells/µm²)

TnA : Level of H3K27me3 or H3K4me2 in the nucleus taeniae (cells/µm²)

**R Code**

# R packages

library(“ggplot2”)

library(“nlme”)

library(“lme4”)

library(“RVAideMemoire”)

library(“car”)

library(“emmeans”)

library(“Rcmdr”)

library(“FactomineR”)

library(“factoextra”)

library(“coin”)

# F2 and F3 offspring’s weight

The same script was used for both generation

mod <- lme(log(weight) ~ treatment + sex + day + treatment:day + treatment:sex + sex:day, random=~1|id, data=data)

plotresid(mod)

Anova(mod)

emmeans(mod, pairwise ~ treatment|sex)

# F2 and F3 offspring’s emotional reactivity

The same script was used for both generation

PCA

usdm::vifstep(data[,5:28],th=5)

F\_PCA <- data[, c("IT\_IND", "IT\_DUREE", "EM\_LTET", "EM\_LATC",

+ "EM\_LCA\_BOX", "EM\_CA\_BOX", "EM\_LCA\_DISPO", "EM\_CA\_DISPO", "EM\_OM", "EM\_OBS",

+ "EM\_EXP", "EM\_PAC", "EM\_PEUR", "EM\_CONF", "NO\_LAPP", "NO\_OM", "NO\_OBS",

+ "NO\_EXP", "NO\_PAC", "NO\_PEUR", "NO\_CONF", "NO\_OBO", "NO\_EXPO", "treatment")]

res <- PCA(F\_PCA, scale.unit=TRUE, ncp=5, quali.sup=c(25: 25), graph =

+ FALSE)

summary(res)

fviz\_eig(res, addlabels = TRUE, ylim = c(0, 50))

Factorial score analysis

mod\_PC1 <- lm(PC1 ~ treatment \* sex, data = data) # For F2 and F3 offspring

plotresid(mod\_PC1)

Anova(mod\_PC1)

mod\_2 <- lm(PC2 ~ treatment \* sex, data = data) # For F2 and F3 offspring

plotresid(mod\_PC2)

Anova(mod\_PC2)

mod\_3 <- lm(PC3 ~ treatment \* sex, data = data) # For F3 offspring only

plotresid(mod\_PC3)

Anova(mod\_PC3)

emmeans(pairwise, mod\_PC3 ~ treatment | sex)

# F1 and F2 eggs characteristics

The same script was used for both generation

Egg weight

wegg\_perm <- oneway\_test(w\_egg ~ treatment, data=data, distribution="exact")

wegg\_perm

Shell weight

wshell\_perm <- oneway\_test(w\_shell ~ treatment, data=data, distribution="exact")

wshell\_perm

Yolk weight

wyolk\_perm <- oneway\_test(w\_yolk ~ treatment, data=data, distribution="exact")

wyolk\_perm

Albumen weight

walbu\_perm <- oneway\_test(w\_albumen ~ treatment, data=data, distribution="exact")

walbu\_perm

Androgens concentration

andro\_yolk\_perm <- oneway\_test(andro\_yolk ~ treatment, data=data, distribution="exact")

andro\_yolk \_perm

AND

andro\_g\_perm <- oneway\_test(andro\_g ~ treatment, data=data, distribution="exact")

andro\_g \_perm

Progesterone concentration

proges\_yolk\_perm <- oneway\_test(proges\_yolk ~ treatment, data=data, distribution="exact")

proges\_yolk \_perm

AND

proges\_g\_perm <- oneway\_test(proges \_g ~ treatment, data=data, distribution="exact")

proges \_g \_perm

# H3K27me3 and H3K4me2 in F3 females’ brains

The same scripts were used for both markers

PVN

pvn\_perm <- oneway\_test(PVN ~ treatment, data=data, distribution="exact")

pvn\_perm

Hippocampus

hp\_perm <- oneway\_test(Hp ~ treatment, data=data, distribution="exact")

hp\_perm

Dorsal amygdala

ad\_perm <- oneway\_test(Ad ~ treatment, data=data, distribution="exact")

ad\_perm

Medial amygdala

am\_perm <- oneway\_test(Am ~ treatment, data=data, distribution="exact")

am\_perm

Intermediate amygdala

ai\_perm <- oneway\_test(Ai ~ treatment, data=data, distribution="exact")

ai\_perm

PoA

poa\_perm <- oneway\_test(PoA ~ treatment, data=data, distribution="exact")

poa\_perm

TnA

tna\_perm <- oneway\_test(TnA ~ treatment, data=data, distribution="exact")

tna\_perm