# Eat yourself sexy: how selective macronutrient intakes influence sexual signal expression in common myna birds

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## ABSTRACT

Foragers have to sample foods in their environment to obtain adequate levels of energy and nutrients depending on their current nutritional needs. The production of carotenoid-based sexual signals in birds likely relies upon a balance of macronutrients and dietary carotenoids. However, the nutritional dynamics associated with carotenoid-based traits remains to be further explored. To address this vexing question, we experimentally manipulate the access of macronutrients and carotenoids in common mynas (Acridotheres tristis) and used signal coloration as a proxy for optimal nutrient balance. Mynas adjusted their macronutrient intake based on sex and also access to dietary carotenoids. Moreover, mynas supplemented with carotenoids had raised levels of plasma carotenoids. Specifically, males, but not females, that had access to carotenoids and could also freely select macronutrients had the highest levels of circulating carotenoids. Intake of dietary carotenoids improved coloration in males but had no effect on female coloration. The overall pattern of dietary choices suggests sex-specific nutritional strategies for this species, with females potentially maximizing nutrient intake for reproductive functions and males selecting macronutrient combinations that increased pigments absorption for signal expression.

### Load packages

library(agricolae)  
library(ade4)  
library(base)  
library(MASS)  
library(lme4)  
library(nlme)  
library(glmm)  
library(coda)  
library(languageR)  
library(nlme)  
library(car)  
library(faraway)  
library(stats)  
library(coin)  
library(survival)   
library(FactoMineR)   
library(sem)  
library(cluster)  
library(pwr)  
library("ggplot2")  
library(multcomp)  
library(lmerTest)  
library(pbkrtest)  
library(mgcv)  
library(fields)  
library(MuMIn)

### MANOVAS

### Gather data

#read in the full dataset  
Diet\_weekly <- read.delim ("Datasets/ Diet\_weekly.txt")

#create subsets

temp=subset(Diet\_weekly, Treatment!="Car")  
temp2=subset(temp, Treatment!="Ctrl")  
temp3=subset(temp2, Treatment!="NullB")  
Diet=subset(temp3, Treatment!="Both")  
temp4=subset(temp2,Treatment!="NullD")  
Both=subset(temp4, Treatment!="Diet")

#create DVs

DV1=cbind(Diet$P.Energy, Diet$L.Energy, Diet$C.Energy)  
DV2=cbind(Both$P.Energy, Both$L.Energy, Both$C.Energy)

### Run MANOVAs

output1=lm(DV1~Treatment, data=Diet)  
manova\_out1=Manova(output1, type="III")  
summary(manova\_out1)

output2=lm(DV2~Treatment, data=Both)  
manova\_out2=Manova(output2, type="III")  
summary(manova\_out2)

### LINEAR MIXED MODELS – NUTRITIONAL ANALYSIS

### Gather data

#read in the full datasets  
Diet\_data <- read.delim ("Datasets/ Diet\_data.txt")

### Run LMMs

#Analysis of difference in % nutrient intake (energy) across treatment/sex

#protein (P)

modelP=lmer(P.Energy.per~Treatment+Sex+ Treatment:Sex + (1|Cohort)

+(1|Bird), REML = TRUE, data=Diet\_data)

plot(modelP, resid(.) ~ fitted(.), abline = 0)  
r.squaredGLMM(modelP)  
anova(modelP,ddf="Kenward-Roger")

#lipid (L)

modelL=lmer(L.Energy.per~Treatment+Sex+ Treatment:Sex + (1|Cohort)

+(1|Bird), REML = TRUE, data=Diet\_data)

plot(modelL, resid(.) ~ fitted(.), abline = 0)  
r.squaredGLMM(modelL)  
anova(modelL,ddf="Kenward-Roger")

#carbohydrate (C)

modelC=lmer(C.Energy.per~Treatment+Sex+ Treatment:Sex + (1|Cohort)

+(1|Bird), REML = TRUE, data=Diet\_data)

plot(modelC, resid(.) ~ fitted(.), abline = 0)  
r.squaredGLMM(modelC)  
anova(modelC,ddf="Kenward-Roger")

### Run Post-hoc comparisons

#create subsets

CTRL=subset(Diet\_data, Treatment=="Ctrl")  
DIET=subset(Diet\_data, Treatment=="Diet")  
CAR=subset(Diet\_data, Treatment=="Car")  
BOTH=subset(Diet\_data, Treatment=="Both")

noCTRL=subset(Diet\_data, Treatment!="Ctrl")  
noDIET=subset(Diet\_data, Treatment!="Diet")  
noCAR=subset(Diet\_data, Treatment!="Car")  
noBOTH=subset(Diet\_data, Treatment!="Both")

Temp=**subset**(Diet\_data, Treatment**!=**"Ctrl")  
BD=**subset**(Temp, Treatment**!=**"Car")  
F=**subset**(BD, Sex**==**"F")  
M=**subset**(BD, Sex**==**"M")

#protein (% P): Treatment

oneway\_test(P.Energy.per ~ Treatment ,

data=subset(noCAR, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(P.Energy.per ~ Treatment ,

data=subset(noDIET, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(P.Energy.per ~ Treatment ,

data=subset(noCAR, Treatment!="Diet"),

distribution=approximate(nresample=1000000))  
oneway\_test(P.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(P.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Car"),

distribution=approximate(nresample=1000000))  
oneway\_test(P.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Diet"),

distribution=approximate(nresample=1000000))

#protein (% P): Sex v Treatment

oneway\_test(P.Energy.per~Sex,

distribution=approximate(nresample=1000000),

teststat="quad", data=DIET)  
oneway\_test(P.Energy.per~Sex,

distribution=approximate(nresample=1000000),

teststat="quad", data=BOTH)  
oneway\_test(P.Energy.per~Treatment,

distribution=approximate(nresample=1000000),

teststat="quad", data=F)  
oneway\_test(P.Energy.per~Treatment,

distribution=approximate(nresample=1000000),

teststat="quad", data=M)

#lipid (% L): Treatment

oneway\_test(L.Energy.per ~ Treatment ,

data=subset(noCAR, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(L.Energy.per ~ Treatment ,

data=subset(noDIET, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(L.Energy.per ~ Treatment ,

data=subset(noCAR, Treatment!="Diet"),

distribution=approximate(nresample=1000000))  
oneway\_test(L.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(L.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Car"),

distribution=approximate(nresample=1000000))  
oneway\_test(L.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Diet"),

distribution=approximate(nresample=1000000))  
  
#carbohydrate (% C): Treatment

oneway\_test(C.Energy.per ~ Treatment ,

data=subset(noCAR, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(C.Energy.per ~ Treatment ,

data=subset(noDIET, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(C.Energy.per ~ Treatment ,

data=subset(noCAR, Treatment!="Diet"),

distribution=approximate(nresample=1000000))  
oneway\_test(C.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Both"),

distribution=approximate(nresample=1000000))  
oneway\_test(C.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Car"),

distribution=approximate(nresample=1000000))  
oneway\_test(C.Energy.per ~ Treatment ,

data=subset(noCTRL, Treatment!="Diet"),

distribution=approximate(nresample=1000000))

#carbohydrate (% C): Treatment v Sex

oneway\_test(C.Energy.per~Sex,

distribution=approximate(nresample=1000000),

teststat="quad", data=DIET)  
oneway\_test(C.Energy.per~Sex,

distribution=approximate(nresample=1000000),

teststat="quad", data=BOTH)  
oneway\_test(C.Energy.per~Treatment,

distribution=approximate(nresample=1000000),

teststat="quad", data=F)  
oneway\_test(C.Energy.per~Treatment,

distribution=approximate(nresample=1000000),

teststat="quad", data=M)

### LINEAR MIXED MODELS – COLORATION AND PLASMA ANALYSIS

### Gather data

#read in the full datasets  
data <- read.delim ("Datasets/ Color\_plasma\_data.txt")

#create subsets

data2=subset(data, Time=="D28")

### Run LMMs

#Analysis of difference in change in coloration score (photochroma) and plasma carotenoids across treatment/sex

#coloration (PhotoC diff)

model2=lmer(PhotoC\_diff~Treatment+Sex+ Treatment:Sex +(1|COHORT),

REML = TRUE, data=photo2)  
plot(model2, resid(.) ~ fitted(.), abline = 0)  
r.squaredGLMM(model2)  
anova(model2,ddf="Kenward-Roger")

#plasma (Plasma diff)

modelCAR=lmer(Plasma\_diff~Treatment+Sex+ Treatment:Sex +(1|COHORT),

REML = TRUE, data=plasma)  
plot(modelCAR, resid(.) ~ fitted(.), abline = 0)  
r.squaredGLMM(modelCAR)  
anova(modelCAR,ddf="Kenward-Roger")

### Run Post-hoc comparisons

#create subsets

noCTRL=subset(data2, Treatment!="CTRL")  
noDIET=subset(data2, Treatment!="DIET")  
noCAR=subset(data2, Treatment!="CAR")  
noBOTH=subset(data2, Treatment!="BOTH")

CTRL=subset(data2, Treatment=="CTRL")  
DIET=subset(data2, Treatment=="DIET")  
CAR=subset(data2, Treatment=="CAR")  
BOTH=subset(data2, Treatment=="BOTH")

dataM=subset(data2, Sex=="M")

noCTRLM=subset(dataM, Treatment!="CTRL")  
noDIETM=subset(dataM, Treatment!="DIET")  
noCARM=subset(dataM, Treatment!="CAR")  
noBOTHM=subset(dataM, Treatment!="BOTH")

dataF=subset(data2, Sex=="F")

noCTRLF=subset(dataF, Treatment!="CTRL")  
noDIETF=subset(dataF, Treatment!="DIET")  
noCARF=subset(dataF, Treatment!="CAR")  
noBOTHF=subset(dataF, Treatment!="BOTH")

#coloration (PhotoC diff): Treatment

oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCAR, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noDIET, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))

oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCAR, Treatment!="DIET"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCTRL, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCTRL, Treatment!="CAR"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCTRL, Treatment!="DIET"),

distribution=approximate(nresample=1000000))

#coloration (PhotoC diff): Treatment v Sex

par(mfrow=c(2,2))  
plot(CTRL$PhotoC\_diff~Sex, data=CTRL)  
plot(DIET$PhotoC\_diff~Sex, data=DIET)  
plot(CAR$PhotoC\_diff~Sex, data=CAR)  
plot(BOTH$PhotoC\_diff~Sex, data=BOTH)  
  
oneway\_test(PhotoC\_diff ~Sex,distribution="exact", teststat="quad",

data=CTRL)  
oneway\_test(PhotoC\_diff ~Sex,distribution="exact", teststat="quad",

data=DIET)  
oneway\_test(PhotoC\_diff ~Sex,distribution="exact", teststat="quad",

data=CAR)  
oneway\_test(PhotoC\_diff ~Sex,distribution="exact", teststat="quad",

data=BOTH)

#coloration (PhotoC diff): Males  
oneway\_test(PhotoC\_diff ~ Treatment,

distribution = approximate(nresample= 1000000),

dataM, teststat="quad")  
  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCARM, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noDIETM, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCARM, Treatment!="DIET"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCTRLM, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCTRLM, Treatment!="CAR"),

distribution=approximate(nresample=1000000))  
oneway\_test(PhotoC\_diff ~ Treatment ,

data=subset(noCTRLM, Treatment!="DIET"),

distribution=approximate(nresample=1000000))

#coloration (PhotoC diff): Females

oneway\_test(PhotoC\_diff ~ Treatment,

distribution = approximate(nresample= 1000000),

dataF, teststat="quad")

#plasma (Plasma diff): Treatment

oneway\_test(Plasma\_diff ~ Treatment ,

data=subset(noCAR, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noDIET, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCAR, Treatment!="DIET"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRL, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRL, Treatment!="CAR"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRL, Treatment!="DIET"),

distribution=approximate(nresample=1000000))

#plasma (Plasma diff): Treatment v Sex  
oneway\_test(Plasma \_diff~Sex,distribution=approximate(nresample=1000000),   
 teststat="quad", data=CTRL)  
oneway\_test(Plasma \_diff~Sex,distribution=approximate(nresample=1000000),   
 teststat="quad", data=DIET)  
oneway\_test(Plasma \_diff~Sex,distribution=approximate(nresample=1000000),   
 teststat="quad", data=CAR)  
oneway\_test(Plasma \_diff~Sex,distribution=approximate(nresample=1000000),   
 teststat="quad", data=BOTH)

#plasma (Plasma diff): Males

oneway\_test(Plasma\_diff ~ Treatment,

distribution = approximate(nresample= 1000000),

dataM, teststat="quad")

oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCARM, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noDIETM, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCARM, Treatment!="DIET"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRLM, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRLM, Treatment!="CAR"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRLM, Treatment!="DIET"),

distribution=approximate(nresample=1000000))

#plasma (Plasma diff): Females

oneway\_test(Plasma\_diff ~ Treatment,

distribution = approximate(nresample= 1000000),

dataF, teststat="quad")

oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCARF, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noDIETF, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCARF, Treatment!="DIET"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRLF, Treatment!="BOTH"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRLF, Treatment!="CAR"),

distribution=approximate(nresample=1000000))  
oneway\_test(Plasma \_diff ~ Treatment ,

data=subset(noCTRLF, Treatment!="DIET"),

distribution=approximate(nresample=1000000))

### GENERALIZED ADDITIVE MODELS – COLORATION AND PLASMA ANALYSIS

### Gather data

#read in the full datasets  
data3 <- read.delim ("Datasets/ Diet\_data.txt")

data4 <- read.delim ("Datasets/ Diet\_plasma\_data.txt")

### Run GAMs

k1<-11  
k2<-11

#coloration

PhotoChroma<-gam(PhotoC~s(P.Energy)+s(L.Energy)+s(C.Energy)+

s(P.Energy,L.Energy) + s(P.Energy,C.Energy)+ s(L.Energy,C.Energy),

gamma=1.0,method="REML",select=TRUE,data=data3)  
summary(PhotoChroma)

#plasma

Plasma<-gam(PlasmaCAR~s(data$P.Energy,k=k1,bs="tp")+

s(data$L.Energy,k=k1,bs="tp")+s(data$C.Energy,k=k1,bs="tp")+

s(data$P.Energy,data$L.Energy,k=k2,bs="tp")+

s(data$P.Energy,data$C.Energy,k=k2,bs="tp")+

s(data$L.Energy,data$C.Energy,k=k2,bs="tp"), gamma=1.0,

method="REML",select=TRUE,data=data4)

summary(Plasma)

### Create spline 2D response surfaces

#coloration

par(mfrow=c(1,1), mar=c(4.5,4.5,2,2))  
surf.te=Tps(cbind(data3$P.Energy,data3$L.Energy),data3$PhotoC,

lambda = 0.1)  
summary(surf.te)  
surf.te.out=predictSurface(surf.te)  
image(surf.te.out, col=tim.colors(128), lwd=5, las=1, font.lab=2,

cex.lab=1.5, cex.axis=1.4, font.axis=1, lab=c(4,4,4), xlim=c(0,250),

ylim=c(0,200), xlab=expression("P.Energy"),

ylab=expression("L.Energy"),main="PhotoChroma",asp=0)  
contour(surf.te.out, lwd=2, labcex=1, nlevels=8, add=T)  
points(data3$P.Energy,data3$L.Energy, cex=0.5, pch=19, col="black")  
  
#plasma

par(mfrow=c(1,1), mar=c(4.5,4.5,2,2))  
surf.te=Tps(cbind(data4$P.Energy,data4$L.Energy),data4$PlasmaCAR,

lambda = 0.1)  
summary(surf.te)  
surf.te.out=predictSurface(surf.te)  
image(surf.te.out, col=tim.colors(128), lwd=5, las=1, font.lab=2,

cex.lab=1.5, cex.axis=1.4, font.axis=1, lab=c(4,4,4), xlim=c(0,450),

ylim=c(0,350), xlab=expression("P.Energy"),

ylab=expression("L.Energy"),main="Plasma CAR", asp=0)  
contour(surf.te.out, lwd=2, labcex=1, nlevels=8, add=T)  
points(data4$P.Energy,data4$L.Energy, cex=0.5, pch=19, col="black")  
  
surf.te=Tps(cbind(data4$C.Energy,data4$L.Energy),data4$PlasmaCAR,

lambda = 0.1)  
summary(surf.te)  
surf.te.out=predictSurface(surf.te)  
image(surf.te.out, col=tim.colors(128), lwd=5, las=1, font.lab=2,

cex.lab=1.5, cex.axis=1.4,font.axis=1, lab=c(4,4,4), xlim=c(0,450),

ylim=c(0,350), xlab=expression("C.Energy"),

ylab=expression("L.Energy"),main="Plasma CAR", asp=0)  
contour(surf.te.out, lwd=2, labcex=1, nlevels=8, add=T)  
points(data4$C.Energy,data4$L.Energy, cex=0.5, pch=19, col="black")

surf.te=Tps(cbind(data4$P.Energy,data4$C.Energy),data4$PlasmaCAR,

lambda = 0.1)  
summary(surf.te)  
surf.te.out=predictSurface(surf.te)  
image(surf.te.out, col=tim.colors(128), lwd=5, las=1, font.lab=2,

cex.lab=1.5, cex.axis=1.4, font.axis=1, lab=c(4,4,4), xlim=c(0,450),

ylim=c(0,450), xlab=expression("P.Energy"),

ylab=expression("C.Energy"),main="Plasma CAR", asp=0)  
contour(surf.te.out, lwd=2, labcex=1, nlevels=8, add=T)  
points(data4$P.Energy,data4$C.Energy, cex=0.5, pch=19, col="black")