R-code for "No evidence of sex allocation among three sympatric plovers in Madagascar that vary in mating system"

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In this document we provide all the necessary code for reproducing the analyses presented in our paper. To access the dataset and Rmarkdown file, please download this GitHub repository. Simply follow the link and click on *Download ZIP* on the right-hand side of the page. An explanation of the files in the repository can be found in the Readme file. Please don't hesitate to contact Luke at `luke.eberhart[at]gmail.com` if you have any questions.

The structure of the code we present here follows the analyses presented in the *Results* section of the paper.

Prerequisites:

- For running the complete code you need a `files` subfolder containing the raw data downloaded from `data` folder provided in the GitHub repository.
- The following packages are needed for analysis and can be easily installed from CRAN by uncommenting the `install.packages` functions:

```
# install.packages("dplyr")
# install.packages("lme4")
# install.packages("ggplot2")
# install.packages("RColorBrewer")
# install.packages("grid")
library(dplyr)
library(lme4)
library(ggplot2)
library(RColorBrewer)
library(grid)
```

Loading and wrangling data

To start, please load the following dataset into your R environment:

- **plover chick data** contains the raw chick data collected from the field. Each row is a single capture of a chick at its nest. Each chick has a unique identifier *chickID* and each brood has a unique identifier *nestID*. The geographic coordinates are also shown as *easting* and *northing* (Coordinate System: UTM Zone 38S Tananarive 1925) which are presented in the map to illustrate the spatial distribution of the nests used in this study. A chick can be one of three plover *species* (Kittlitz's, Madagascar, or White-fronted). *year* indicates the year during which an individual was captured and *dateinseason* indicates the number of days since the start of the breeding season that an individual hatched. The dummy variables *male* and *female* indicate the molecular sex-type of an individual with "1" signifing the sex.

```r
plover_chick <-
  read.table(file = "data/plover_chick_data.txt",
             header = TRUE,
             stringsAsFactors = FALSE,
             sep = "\t")
```

Define the variables of the `plover_chick` correctly: `easting` and `northing` need to recognized as numeric, and `chickID`, `nestID`, `species`, and `year` need to recognized as factors.

```r
plover_chick[,c("easting", "northing")] <-
  lapply(plover_chick[,c("easting", "northing")], as.numeric)

plover_chick[,c("chickID", "nestID", "species", "year")] <-
  lapply(plover_chick[,c("chickID", "nestID", "species", "year")], as.factor)
```

Summarize the `plover_chick` data such that each row represents data for a nest.

```r
plover_nest <-
  plover_chick %>%
  dplyr::group_by(species, year, nestID) %>%
  dplyr::summarise(no_males = sum(male),
                   no_females = sum(female),
                   Year = first(year),
                   dateinseason = first(dateinseason),
                   easting = first(easting),
                   northing = first(northing),
                   brood_size = no_males + no_females,
                   prop_male = no_males / brood_size)
```

Do hatching sex ratios deviate from parity?

Linear regression mixed models for each species predicting deviation in hatching sex ratio while controlling for non-independence in siblings (i.e. random effect: `nestID`).

- **Kittlitz's plover**

```r
KiP_HSR_model <- lme4::glmer(cbind(no_males, no_females) ~ (1| nestID),
                             data = dplyr::filter(plover_nest, species == "Kittlitz's"),
                             family = binomial)
summary(KiP_HSR_model)
#> Generalized linear mixed model fit by maximum likelihood (Laplace
#>   Approximation) [glmerMod]
#>  Family: binomial  ( logit )
#> Formula: cbind(no_males, no_females) ~ (1 | nestID)
#>    Data: dplyr::filter(plover_nest, species == "Kittlitz's")
#>
#>      AIC      BIC   logLik deviance df.resid
#>    153.3    157.8    -74.6    149.3       70
#>
```

```
#> Scaled residuals:
#>     Min      1Q   Median       3Q      Max
#> -1.49509 -0.07869 -0.07869  1.33771  1.33771
#>
#> Random effects:
#>  Groups Name        Variance Std.Dev.
#>  nestID (Intercept) 0        0
#> Number of obs: 72, groups:  nestID, 72
#>
#> Fixed effects:
#>             Estimate Std. Error z value Pr(>|z|)
#> (Intercept)   0.1112     0.1669   0.666    0.505
```

- **Madagascar plover**

```
MP_HSR_model <- lme4::glmer(cbind(no_males, no_females) ~ (1| nestID),
                      data = dplyr::filter(plover_nest, species == "Madagascar"),
                      family = binomial)
summary(MP_HSR_model)
#> Generalized linear mixed model fit by maximum likelihood (Laplace
#>   Approximation) [glmerMod]
#>  Family: binomial  ( logit )
#> Formula: cbind(no_males, no_females) ~ (1 | nestID)
#>    Data: dplyr::filter(plover_nest, species == "Madagascar")
#>
#>      AIC      BIC   logLik deviance df.resid
#>     21.8     22.5     -8.9     17.8        9
#>
#> Scaled residuals:
#>     Min      1Q  Median       3Q      Max
#> -0.4009 -0.4009 -0.4009   0.3341   1.0690
#>
#> Random effects:
#>  Groups Name        Variance Std.Dev.
#>  nestID (Intercept) 0        0
#> Number of obs: 11, groups:  nestID, 11
#>
#> Fixed effects:
#>             Estimate Std. Error z value Pr(>|z|)
#> (Intercept)   0.5596     0.4432   1.263    0.207
```

- **White-fronted plover**

```
WfP_HSR_model <- lme4::glmer(cbind(no_males, no_females) ~ (1| nestID),
                      data = dplyr::filter(plover_nest, species == "White-fronted"),
                      family = binomial)
summary(WfP_HSR_model)
#> Generalized linear mixed model fit by maximum likelihood (Laplace
#>   Approximation) [glmerMod]
#>  Family: binomial  ( logit )
#> Formula: cbind(no_males, no_females) ~ (1 | nestID)
#>    Data: dplyr::filter(plover_nest, species == "White-fronted")
```

```
#> 
#>      AIC      BIC   logLik deviance df.resid
#>     34.3     35.4    -15.1     30.3       11
#> 
#> Scaled residuals:
#>     Min      1Q  Median      3Q     Max
#> -1.1406 -0.2352 -0.2352  0.7478  0.8308
#> 
#> Random effects:
#>  Groups Name        Variance Std.Dev.
#>  nestID (Intercept) 1.57     1.253
#> Number of obs: 13, groups:  nestID, 13
#> 
#> Fixed effects:
#>             Estimate Std. Error z value Pr(>|z|)
#> (Intercept)   0.5887     0.5923   0.994     0.32
```

Summarize the `plover_nest` data such that each row represents a species.

```r
plover_summary <-
  plover_nest %>%
  dplyr::group_by(species) %>%
  dplyr::summarise(n_chicks = sum(brood_size),
                   n_nests = length(prop_male),
                   mean = mean(prop_male),
                   SE = sd(prop_male) / sqrt(length(prop_male)),
                   lower_CI =
                     mean(prop_male) - (sd(prop_male) / sqrt(length(prop_male)) * 1.96),
                   upper_CI =
                     mean(prop_male) + (sd(prop_male) / sqrt(length(prop_male)) * 1.96))
```

Plot the species summary.

```r
# draw the blank plot for the background
Figure_1_blank <-
  ggplot2::ggplot(data = plover_summary, aes(y = mean, x = species)) +
          theme_bw() +
          annotate("rect", xmin = 0, xmax = 6, ymin = 0, ymax = 0.5, alpha = 0.7,
                   fill = RColorBrewer::brewer.pal(8, "Dark2")[c(1)]) +
          annotate("rect", xmin = 0, xmax = 6, ymin = 0.5, ymax = 1, alpha = 0.7,
                   fill = RColorBrewer::brewer.pal(8, "Dark2")[c(2)]) +
            theme(text = element_text(colour = "white"),
                  legend.position = "none",
                  legend.background = element_rect(fill = NA),
                  axis.title.y = element_text(size = 12, margin = margin(0, 10, 0, 0)),
                  axis.text.y  = element_text(size = 10),
                  axis.title.x = element_text(size = 12, margin = margin(20, 0, 0, 0)),
                  axis.text.x  = element_text(size = 10),
                  axis.ticks.x = element_blank(),
                  axis.ticks.y = element_line(size = 0.5, colour = "white"),
                  axis.ticks.length = unit(0.2, "cm"),
                  panel.grid.major = element_blank(),
                  panel.grid.minor = element_blank(),
```

```r
                      panel.border = element_blank(),
                      panel.margin = unit(0.75, "lines"),
                      plot.margin = unit(c(0.5, 1.5, 0.5 ,0), "cm")) +
                scale_y_continuous(limits = c(0, 1), expand = c(0, 0)) +
                scale_x_continuous(limits=c(0, 6),breaks=c(0, 1, 2), expand = c(0, 0)) +
                xlab("Plover species") +
                ylab("Hatching sex ratio (proportion male)")


# draw the plot with the data
Figure_1 <-
  ggplot2::ggplot(data = plover_summary, aes(y = mean, x = species)) +
            theme_bw() +
            geom_pointrange(aes(ymin = lower_CI, ymax = upper_CI)) +
            theme(legend.position = "none",
                  panel.background = element_rect(fill = "transparent",colour = NA),
                  plot.background = element_rect(fill = "transparent",colour = NA),
                  axis.title.y = element_text(size = 12, margin = margin(0, 10, 0, 0)),
                  axis.text.y  = element_text(size = 10),
                  axis.title.x = element_text(size = 12, margin = margin(20, 0, 0, 0)),
                  axis.text.x  = element_text(size = 10),
                  axis.ticks.x = element_blank(),
                  axis.ticks.y = element_line(size = 0.5, colour = "grey40"),
                  axis.ticks.length = unit(0.2, "cm"),
                  panel.grid.major = element_blank(),
                  panel.grid.minor = element_blank(),
                  panel.border = element_blank(),
                  panel.margin = unit(0.75, "lines"),
                  plot.margin = unit(c(0.5, 1.5, 0.5, 0), "cm")) +
            scale_y_continuous(limits = c(0, 1), expand = c(0, 0)) +
            xlab("Plover species") +
            ylab("Hatching sex ratio (proportion male)")


# Print the two layers
grid::pushViewport(
  grid::viewport(
    layout = grid::grid.layout(1, 1, widths = unit(1, "npc"))))
print(Figure_1_blank, newpage = FALSE)
print(Figure_1, newpage = FALSE)
grid::popViewport()
```
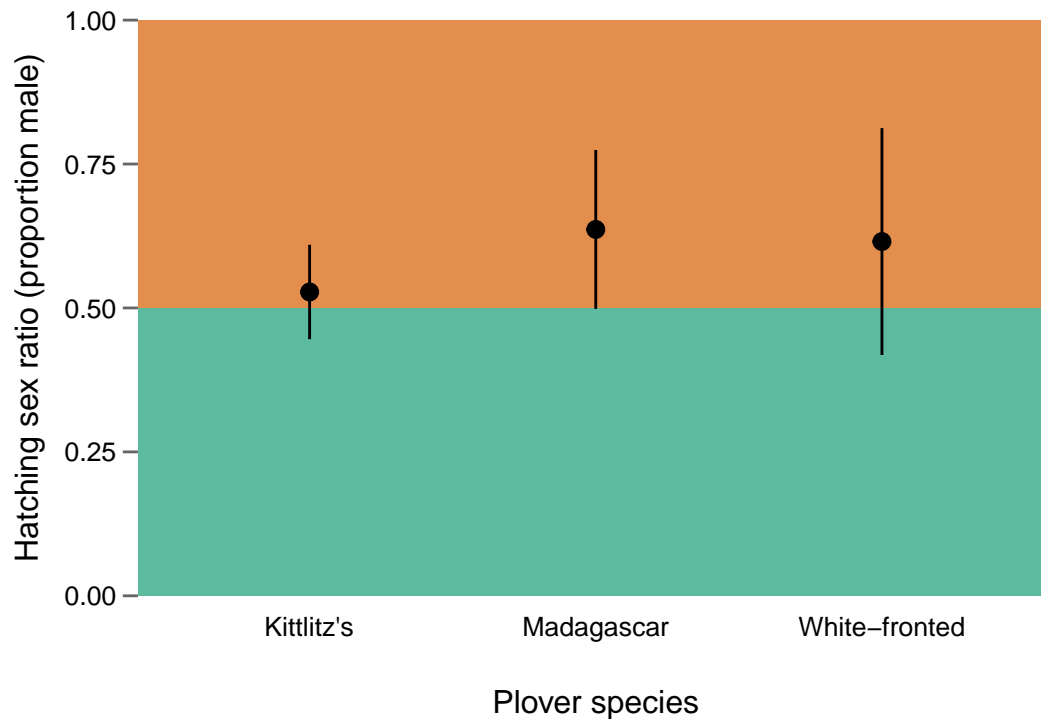
Does hatching sex ratio vary seasonaly or annually?

Linear regression mixed models for each species predicting annual and seasonal deviations in hatching sex ratio while controlling for non-independence in siblings (i.e. random effect: `nestID`).

- **Kittlitz's plover**

```r
# Annual model
KiP_HSR_year <-
  lme4::glmer(male ~ (1| nestID) + year,
              data = dplyr::filter(plover_chick, species == "Kittlitz's"),
              family = binomial)

# Seasonal model
KiP_HSR_season <-
  lme4::glmer(male ~ (1| nestID) + dateinseason,
              data = dplyr::filter(plover_chick, species == "Kittlitz's"),
              family = binomial)

# No temporal effect model
KiP_HSR_null <-
  lme4::glmer(male ~ (1| nestID),
              data = dplyr::filter(plover_chick, species == "Kittlitz's"),
              family = binomial)

# Assess the effect of year
anova(KiP_HSR_year, KiP_HSR_null)
```

```
#> Data: dplyr::filter(plover_chick, species == "Kittlitz's")
#> Models:
#> KiP_HSR_null: male ~ (1 | nestID)
#> KiP_HSR_year: male ~ (1 | nestID) + year
#>              Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
#> KiP_HSR_null  2 203.18 209.12 -99.591   199.18
#> KiP_HSR_year  5 205.68 220.53 -97.840   195.68 3.5016      3     0.3206

# Assess the effect of season
anova(KiP_HSR_season, KiP_HSR_null)
#> Data: dplyr::filter(plover_chick, species == "Kittlitz's")
#> Models:
#> KiP_HSR_null: male ~ (1 | nestID)
#> KiP_HSR_season: male ~ (1 | nestID) + dateinseason
#>                Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
#> KiP_HSR_null    2 203.18 209.12 -99.591   199.18
#> KiP_HSR_season  3 204.62 213.53 -99.312   198.62 0.5587      1     0.4548
```

- **Madagascar plover**

```
# Annual model
MP_HSR_year <-
  lme4::glmer(male ~ (1| nestID) + year,
              data = dplyr::filter(plover_chick, species == "Madagascar"),
              family = binomial)

# Seasonal model
MP_HSR_season <-
  lme4::glmer(male ~ (1| nestID) + dateinseason,
              data = dplyr::filter(plover_chick, species == "Madagascar"),
              family = binomial)

# No temporal effect model
MP_HSR_null <-
  lme4::glmer(male ~ (1| nestID),
              data = dplyr::filter(plover_chick, species == "Madagascar"),
              family = binomial)

# Assess the effect of year
anova(MP_HSR_year, MP_HSR_null)
#> Data: dplyr::filter(plover_chick, species == "Madagascar")
#> Models:
#> MP_HSR_null: male ~ (1 | nestID)
#> MP_HSR_year: male ~ (1 | nestID) + year
#>             Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
#> MP_HSR_null  2 32.841 35.023 -14.421   28.841
#> MP_HSR_year  5 37.253 42.708 -13.626   27.253 1.5886      3     0.662

# Assess the effect of season
anova(MP_HSR_season, MP_HSR_null)
#> Data: dplyr::filter(plover_chick, species == "Madagascar")
#> Models:
#> MP_HSR_null: male ~ (1 | nestID)
```

```
#> MP_HSR_season: male ~ (1 | nestID) + dateinseason
#>                Df    AIC    BIC   logLik deviance  Chisq Chi Df Pr(>Chisq)
#> MP_HSR_null     2 32.841 35.023 -14.421   28.841
#> MP_HSR_season   3 34.468 37.741 -14.234   28.468 0.3735      1     0.5411
```

- **White-fronted plover**

```r
# Annual model
WfP_HSR_year <-
  lme4::glmer(male ~ (1| nestID) + year,
              data = dplyr::filter(plover_chick, species == "White-fronted"),
              family = binomial)

# Seasonal model
WfP_HSR_season <-
  lme4::glmer(male ~ (1| nestID) + dateinseason,
              data = dplyr::filter(plover_chick, species == "White-fronted"),
              family = binomial)

# No temporal effect model
WfP_HSR_null <-
  lme4::glmer(male ~ (1| nestID),
              data = dplyr::filter(plover_chick, species == "White-fronted"),
              family = binomial)

# Assess the effect of year
anova(WfP_HSR_year, WfP_HSR_null)
#> Data: dplyr::filter(plover_chick, species == "White-fronted")
#> Models:
#> WfP_HSR_null: male ~ (1 | nestID)
#> WfP_HSR_year: male ~ (1 | nestID) + year
#>               Df    AIC    BIC   logLik deviance  Chisq Chi Df Pr(>Chisq)
#> WfP_HSR_null   2 42.606 45.408 -19.303   38.606
#> WfP_HSR_year   4 43.114 48.718 -17.557   35.114 3.4922      2     0.1745

# Assess the effect of season
anova(WfP_HSR_season, WfP_HSR_null)
#> Data: dplyr::filter(plover_chick, species == "White-fronted")
#> Models:
#> WfP_HSR_null: male ~ (1 | nestID)
#> WfP_HSR_season: male ~ (1 | nestID) + dateinseason
#>                 Df    AIC    BIC   logLik deviance  Chisq Chi Df Pr(>Chisq)
#> WfP_HSR_null     2 42.606 45.408 -19.303   38.606
#> WfP_HSR_season   3 44.107 48.310 -19.053   38.107 0.4992      1     0.4798
```