## **READ ME**

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#### **Location & dates of data collection**

Behavioural observations were conducted at Witley Common, Surrey, UK (51°09'04"N 0°40'53"W). The observation area was a 10 x 3 m section of a blind-ended sandy path unused by humans, within heathland dominated by heather. The nesting area was observed continuously by 2-3 people simultaneously between approximately 09.00 and 19.00 on all days with weather suitable for wasp activity.

2012: 23rd July - 15th August

 $2013: 5^{th} - 30^{th} \ July \\ 2014: 1^{st} - 24^{th} \ July$ 

# Methods of data processing and analysis

Data were analyzed using R version 4.0.3 (R Core Team, 2020). Means are reported  $\pm$ one standard error, and statistical significance was assessed at the P=0.05 level. Note that in all data files, variable names and R script we use the term 'parasite' interchangeably with 'foreign female'.

# **File Directory:**

Data files organised into the corresponding results sections:

#### **Provisioning by foreign females**

- 1. NumberOfMeetingsData.csv
- 2. NumberofPreyProvisionedData.csv
- 3. OffspringWeightData.csv

# The classic 'No return' strategy performs no better than the observed strategy

4. AlternativeStrategiesData

# Mistaken provisioning

5. MistakenProvisioningData.csv

# **Genetic relatedness**

6. Relatedness&BehaviourData.csv

## Are foreign females parasites?

7. HostParasitePayoffsData

# Foreign females are winning the evolutionary conflict

- 8. WindowOfConflictData
- 9. PlotDataFig4

# **File descriptions:**

# CuckoosThatCareRScript.R

R script for all statistical analyses, including code to make figures.

# NumberOfMeetingsData

Results section in paper: 'Provisioning by foreign females'

Dataset showing the number of times females met at multi-female nests, and whether both or just one female provisioned.

- Result in paper: 'Host and foreign female met at only 33% of nests where both females remained alive, and the number of meetings did not influence whether both then provisioned (Binomial GLM,  $X^2_1$ =0.6, P=0.44, n=25).' Explanation of variables:
- Year: Year of data collection
- Nest: number of nest
- Date: day number, starts from 0 at start (e.g. 29<sup>th</sup> June in 2013)

- OwnerID: The unique colour ID of owner, e.g. YWB = yellow, white, blue.
- ParaID: The unique colour ID of parasite.
- Relatedness: relatedness value between the two females (calculated using a Monte Carlo method see methods section of manuscript)
- NumFemalesThatProvision: Number of females that provisioned
- NumFemsAlive: Number of females that are alive (thus able to provision)
- Used cbind function in R to combine these 2 values into the proportion of females that provisioned prey at multi-female nests (that were alive to do so) = y variable for this analysis.
- NumMeetings: the number of times owner and parasite met at the nest

# NumberofPreyProvisionedData

Results section in paper: 'Provisioning by foreign females'

Dataset showing the number of prey provisioned according to female roles.

Results in the paper: 'When they did provision, foreign females provided the same number of prey as hosts, and mothers provided the same number of prey as non-mothers (hosts:  $2.68 \pm 0.34$  prey, n=22; foreign females:  $3.25 \pm 0.35$  SE, n=20, Poisson GLM,  $X^2_1$ =1.14, P=0.28; mothers:  $3.33 \pm 0.37$  prey, n=21; non-mothers: 2.57 + 0.31, n=22, Poisson GLM,  $X^2_1$ =2.07, P=0.15).'

#### Explanation of variables:

- Year: of data collection
- Nest: number of nest
- Date: starts from 0 at start (e.g. 29th June in 2013)
- PreyProvisioned: total number of prey items each larva received at a nest
- FemaleSize: focal female size in mm
- Role: whether focal female was in the role of 'parasite' or 'owner'
- MotherOfOffspring: whether focal female was genotyped to be the mother of final offspring (1=mother; 0=non-mother)
- FocalFemaleID: The unique colour ID of owner or parasite

# OffspringWeightData

Results section in paper: 'Provisioning by foreign females'
Dataset showing offspring weight, whether the nest was 'single' or 'joint' provisioned, and the size (in mm) of mothers.

Result in the paper from this dataset:

➤ Offspring at joint-provisioned nests were significantly heavier at the end of feeding than offspring with only a single provisioner (LMER, X21=19.5, P=0.000001; offspring sex X21=3.8, P=0.05).

### Explanation of variables:

- Nest: number of nest
- Date: starts from 0 at start (29th June in 2013)
- OffspringWeight: weight of prepupa in grams

- SinglyOrJointlyProvisioned: whether the nest was provisioned by just one female or by both females
- OffspringSex: M = male; F = female
- MotherOfOffspring: the unique colour ID of the genotyped mother of offspring. Used as a random effect in lmer.
- MotherSize: size of mother in mm
- Outliers: for this analysis, in order to meet the assumption of normality, 6 outliers with high Cook's distance were excluded, these have a '1' in the column 'Outliers'. However, results were similar when outliers were included, and whether the nest was joint or single provisioned still had a significant effect on prepupal weight (LMER, X21=7.6, P=0.006; offspring sex X21=4.4, P=0.04, n=85).
- Note: including outliers increased the significance of offspring sex (including outliers p = 0.036; excluding outliers p = 0.051), however this is not a focal result and did not change the main finding that joint provisioned nests are heavier than single provisioned nests.

# AlternativeStrategiesData

Results section in paper: 'The classic 'No return' strategy performs no better than the observed strategy'

Dataset showing the payoffs from the observed strategy and alternative strategies females could use. This is the dataset used to make Figure 2.

Results in the paper from this dataset:

- Payoffs from the No Return strategy did not differ from observed payoffs (paired Wilcoxon test, V=286, P=0.64).
- ➤ The second hypothetical strategy, Conditional Provision, where foreign females provision only if the host nest is empty when first discovered, performed slightly better than the observed strategy (Fig. 2*A*,*C*; paired Wilcoxon test, *V*=111, *P*=0.04).

### Explanation of variables:

- ParasiteID unique colour ID of parasite.
- NestEmptyWhenEntered 1=empty; 0=contained host offspring
- ObservedPayoff Calculated payoff for observed behaviour of parasites
- NoReturnPayoff Calculated payoff if parasites use the classic strategy of never returning to host nest after laying egg.
- CondProvisionPayoff Calculated payoff if parasites use the strategy of provisioning only if the host nest was empty when first discovered.
- NestNumGrouped orders nests for Fig.2
- ShadingInFig2NoRet codes the line types for Fig.2 for difference between No Return & Observed payoffs
- ShadingInFig2CProv codes the line types for Fig.2 for difference between Conditional Provision & Observed payoffs

# MistakenProvisioningData

Results section in paper: 'Mistaken provisioning'

Dataset showing whether females eject host offspring after entering a foreign nest or if they mistakenly provision it, and whether this depends on spatial distance between nests.

Result in paper: 'The distance between a foreign nest and the nest of her own where she had last been active was shorter for foreign nests that a focal female mistakenly provisioned (152+32 cm, range 1-348) than for nests where she ejected the host offspring (240+34 cm, range 18-552; Binomial GLMM: X21=4.9, N=33, P=0.028).'

# Explanation of variables:

- Mistake1Eject0: After entering a foreign nest, females may:
  - 1 = mistakenly provision another female's offspring
  - 0 = eject the host's egg and lay their own.
- Female: unique colour ID of female, used as a random effect in glmer.
- Nest: number of nest
- Date: starts from 0 at start (e.g., 29th June in 2013)
- Relatedness: relatedness value between the two females (calculated using a Monte Carlo method see methods section of manuscript)
- DistanceToPreviousNest: distance (in cm) from the focal nest to the foreign female's own nest where she was last active. This variable was scaled to fit in the model (see R script)
- AgeOfLarva: age of larva in hours

## Relatedness&BehaviourData

Results section in paper: 'Genetic relatedness'

Dataset showing whether foreign females discard a host female's egg or not after entering foreign nests, the IDs of parasites/owners and their relatedness.

➤ Result in paper: 'After a female entered a foreign nest containing an offspring, her relatedness to the nest owner did not differ in cases where she ejected the offspring (n=39, r=0.008±0.03) versus cases where she re-closed the nest without ejecting (n=24, r=-0.005±0.03; X²₁=0.10, P=0.75; or X²₁=0.19, N=48, P=0.67 excluding mistaken provisioners).'

## Explanation of variables:

- Nest: number of nest
- EjectsOrNot:
  - 1 = ejects host egg
  - 0 = foreign female only entered the nest, did not eject host egg.
- EjectsOrNotExcludingMistakenProvisioners: This is the same as the above measure, however it excludes 'mistaken provisioners' so n = 48. Mistaken provisioners are females who provision another female's offspring.
- Relatedness: relatedness value between the two females (calculated using a Monte Carlo method see methods section of manuscript)
- Date: starts from 0 at start (e.g., 29th June in 2013)
- Parasite: The unique colour ID of parasite
- Owner: The unique colour ID of owner

# HostParasitePayoffsData

Results section in paper: 'Are foreign females parasites?'

Dataset showing the mean payoff scores for unparasitized females, hosts, and parasites.

- Results in paper:
- ➤ On average, hosts therefore had significantly lower payoffs when their nests were utilized by conspecifics, forfeiting approximately half an offspring (n=46 females that acted as hosts versus n=66 that had unparasitized nests; Wilcoxon test, W=503, P=1.9 x 10<sup>-9</sup>).
- ➤ Wasps obtained significantly higher payoffs as foreign females than when they initiated their own nests (Fig. 3, *N*=41 females that acted as foreign females versus *N*=73 single females/hosts combined, Wilcoxon test, *W*=2240, P=0.00001).

### Explanation of variables:

- FemaleID unique colour ID of female
- MeanPayoffWhenHost Mean payoff for female in the role of 'host'
- MeanPayoffWhenUnparasitized Mean payoff for a female when unparasitized
- MeanPayoffWhenParasite Mean payoff for female in the role of 'parasite'
- MeanPayoffWhenUnparasitizedOrHost Combined mean payoffs for unparasitized females and hosts.

### WindowOfConflictData

Results section in paper: 'Foreign females are winning the evolutionary conflict' Dataset showing the observed payoff scores for hosts and parasites, their payoffs if they always produced the final offspring and payoffs if they never provisioned.

- Result in paper: In the window of conflict, hosts lie only 22% of the way towards the optimum from the worst-case scenario where they carry out all of the post-hatch provisioning but fail to produce an offspring (open symbols in Fig. 4: payoff 0.046±0.07, n=26, interquartile range 0.22).
- ➤ In contrast, foreign females lie 65% of the way towards the optimum, scoring  $0.57\pm0.07$  (n=28, interquartile range 0.95; Wilcoxon test, W=114.5, P=0.001). Explanation of variables:
  - HostOrParasite whether female was in the role of host or parasite
  - FocalFemaleID unique colour ID of female
  - ObservedPayoff payoff from observed behaviour
  - PayoffAlwaysProduceOffspring payoff if we assume that the focal female always produces the final offspring
  - PayoffExcludingProvisioningCosts –payoff if we assume that the focal female's partner in the interaction does all of the provisioning
  - To facilitate paired tests, the following 9 columns repeat the same data, but with separate columns for Hosts and Parasites, with the host and parasite from a given interaction in the same row. Two interactions involved 2 parasites each, and a random one of these has been excluded from the data for paired tests.

# PlotDataFig4

Dataset containing means and standard errors to make the plot in Figure 4. This dataset contains summary data from the above 'WindowOfConflictData' file