GLMM analysis of feeding behaviours in *An. stephensi* females (figure 1D, E)

Data from D0 (day of emergence) were excluded from the GLMM analysis because newly emerged females show no feeding behaviour on this day, resulting in no variance in the response variable to model.

Model 1: Whether the experimental groups differ in blood-feeding behaviour across age?

Random variables: BioID (replicate identity), larval.batch (larval batch)

Fixed variables: Day(age), Group (co-housed vs virgin)

```
model1 <- glmer(Bloodfed_binary ~ Day * Group + (1|BioID) + (1|larval.batch),
        data = mosquito_data_filtered,
        family = binomial)
```

Model summary:

Characteristic	Odds	Probability (%)	OR[1]	95% CI	p-value
Day					
D1	1.41	58.5	—	—	
D2	2.67	72.8	1.90**	1.29, 2.81	**0.001**
D3	4.87	83.0	3.47***	2.25, 5.35	**<0.001**
D4	6.35	86.4	4.52***	2.86, 7.13	**<0.001**
D5	4.64	82.3	3.30***	2.15, 5.08	**<0.001**
Group					
co-housed	1.41	58.5	—	—	
virgin	1.20	54.5	0.86	0.59, 1.23	0.403
Day * Group					
D2 * virgin	2.44	70.9	1.74	0.99, 3.05	0.053
D3 * virgin	0.95	48.7	0.68	0.38, 1.21	0.189
D4 * virgin	0.56	35.9	0.40**	0.22, 0.72	**0.002**
D5 * virgin	0.90	47.4	0.64	0.36, 1.14	0.130

[1] *p<0.05; **p<0.01; ***p<0.001

Abbreviations: CI = Confidence Interval, OR = Odds Ratio

Post-hoc analysis via estimated marginal means (EM means)

1) Age-effects within each group (p-value adjustment: Tukey's method)

Group = co-housed:

contrast	estimate	SE	df	z.ratio	p.value
D1 - D2	-0.6423	0.199	Inf	-3.234	0.0107
D1 - D3	-1.2432	0.221	Inf	-5.614	<.0001
D1 - D4	-1.5085	0.233	Inf	-6.479	<.0001
D1 - D5	-1.1946	0.220	Inf	-5.429	<.0001
D2 - D3	-0.6009	0.228	Inf	-2.638	0.0637
D2 - D4	-0.8662	0.242	Inf	-3.577	0.0032
D2 - D5	-0.5523	0.229	Inf	-2.416	0.1111
D3 - D4	-0.2653	0.261	Inf	-1.018	0.8473
D3 - D5	0.0485	0.248	Inf	0.196	0.9997
D4 - D5	0.3138	0.262	Inf	1.199	0.7521

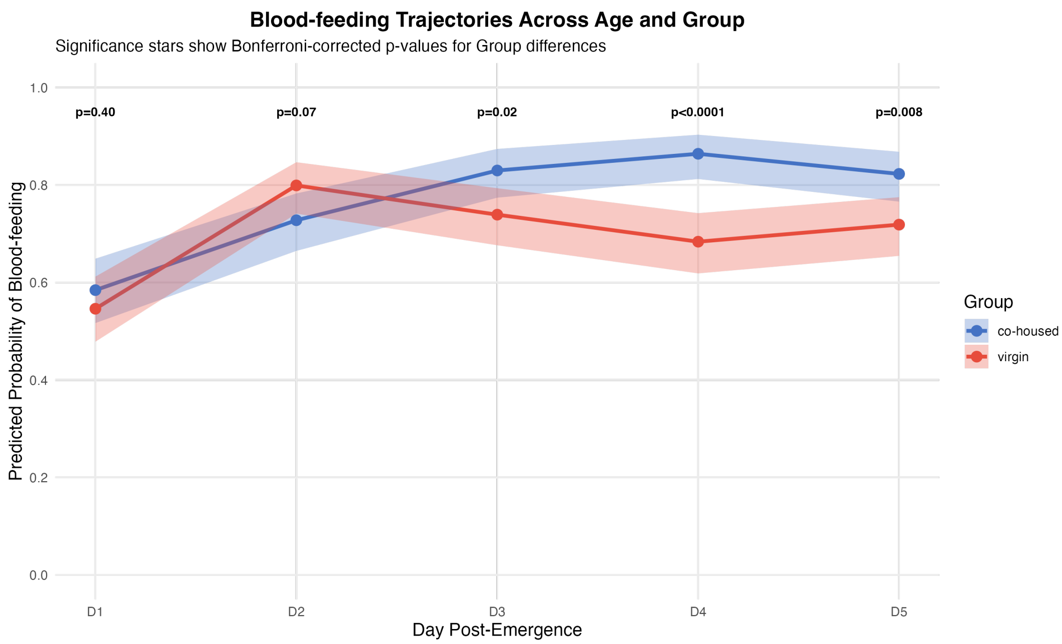
Group = virgin:

contrast	estimate	SE	df	z.ratio	p.value
D1 - D2	-1.1958	0.210	Inf	-5.697	<.0001
D1 - D3	-0.8564	0.201	Inf	-4.262	0.0002
D1 - D4	-0.5857	0.192	Inf	-3.050	0.0194
D1 - D5	-0.7529	0.199	Inf	-3.791	0.0014
D2 - D3	0.3394	0.220	Inf	1.542	0.5353
D2 - D4	0.6100	0.216	Inf	2.826	0.0379
D2 - D5	0.4429	0.221	Inf	2.007	0.2625
D3 - D4	0.2706	0.207	Inf	1.308	0.6859
D3 - D5	0.1035	0.211	Inf	0.491	0.9882
D4 - D5	-0.1671	0.207	Inf	-0.806	0.9288

Results are given on the log odds ratio (not the response) scale.
P value adjustment: tukey method for comparing a family of 5 estimates

2) Pair-wise comparison at each day (p-value adjustment: Bonferroni method)

```
Day = D1:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin    0.156 0.186 Inf   0.836  0.4034

Day = D2:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin   -0.398 0.217 Inf  -1.830  0.0672

Day = D3:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin    0.542 0.228 Inf   2.376  0.0175

Day = D4:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin    1.078 0.236 Inf   4.570  <.0001

Day = D5:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin    0.597 0.225 Inf   2.656  0.0079

Results are given on the log odds ratio (not the response) scale.
```



__**Conclusions:**__ Blood-feeding probability of co-housed females increases with age. This trend is weaker in virgins.

Mated females feed significantly higher than virgins as they age (D3-D5), the differences being most pronounced on D4.

Model 2: Effect of mating on the blood-feeding behaviour of co-housed females

Random variables: BioID (replicate identity)

Fixed variables: Day(age), Mating status (yes or no)

```
model2 <- glmer(Bloodfed_binary ~ Day * Mating + (1|BioID),
            data = mated_only,
            family = binomial)
```

Model summary:

Characteristic	Odds	Probability (%)	OR[1]	95% CI	p-value
Day					
D1	1.37	57.8	—	—	
D2	2.14	68.2	1.56	1.00, 2.46	0.052
D3	2.23	69.0	1.63	0.87, 3.06	0.130
D4	4.23	80.9	3.09*	1.21, 7.88	**0.018**
D5	1.54	60.6	1.12	0.18, 6.89	0.902
Mating					
n			—	—	
y	2.63	72.5	1.92	0.49, 7.46	0.347
Day * Mating					
D2 * y	1.22	55.0	0.89	0.20, 3.92	0.878
D3 * y	2.18	68.6	1.59	0.34, 7.42	0.555
D4 * y	1.14	53.3	0.83	0.16, 4.42	0.827
D5 * y	2.28	69.5	1.67	0.17, 16.2	0.660

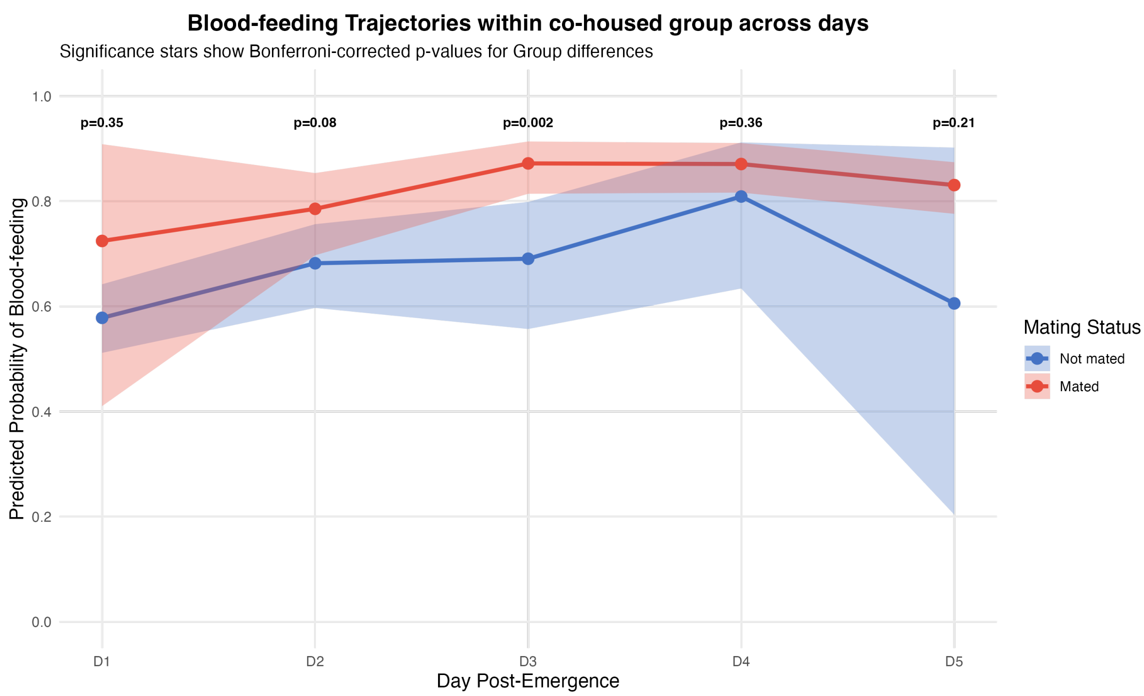
[1] *p<0.05; **p<0.01; ***p<0.001

Abbreviations: CI = Confidence Interval, OR = Odds Ratio

<u>Post-hoc analysis via estimated marginal means (EM means)</u>

Effect of mating (y) on each day (p-value adjustment: Bonferroni method)

```
Day = D1:
 contrast estimate    SE  df z.ratio p.value
 y - n       0.651 0.693 Inf   0.939  0.3475

Day = D2:
 contrast estimate    SE  df z.ratio p.value
 y - n       0.535 0.301 Inf   1.777  0.0756

Day = D3:
 contrast estimate    SE  df z.ratio p.value
 y - n       1.115 0.368 Inf   3.027  0.0025

Day = D4:
 contrast estimate    SE  df z.ratio p.value
 y - n       0.464 0.504 Inf   0.920  0.3575

Day = D5:
 contrast estimate    SE  df z.ratio p.value
 y - n       1.162 0.934 Inf   1.245  0.2133

Results are given on the log odds ratio (not the response) scale.
```



<u>Conclusions:</u> Within co-housed females, mating status shows only a brief, significant effect on blood-feeding on D3. The pronounced differences observed in the blood-feeding behaviour of virgin and co-housed females on Days 4-5 (model 1) appear to be mediated by male presence rather than mating per se, as mated and unmated females within the co-housed group show no significant differences during these time points.

Model 3: Whether the experimental groups differ in their second-blood meal, across age?

Random variables: BioID (replicate identity)

Fixed variables: Day(age), Group (co-housed vs virgin)

```
model3 <- glmer(Bloodfed_binary ~ Day * Group + (1|BioID),
            data = mosquito_data,
            family = binomial)
```

Model summary:

Characteristic	Odds	Probability (%)	OR[1]	95% CI	p-value
Day					
DB1	0.51	33.8	—	—	
DB2	0.21	17.4	0.41***	0.29, 0.60	**<0.001**
DB3	0.65	39.4	1.28	0.92, 1.78	0.143
DB4	0.40	28.6	0.78	0.55, 1.10	0.154
Group					
co-housed	0.51	33.8	—	—	
virgin	4.35	81.3	8.58***	5.81, 12.7	**<0.001**
Day * Group					
DB2 * virgin	1.47	59.5	2.90***	1.67, 5.02	**<0.001**
DB3 * virgin	0.47	32.0	0.92	0.54, 1.55	0.748
DB4 * virgin	0.97	49.2	1.91*	1.11, 3.28	**0.020**

[1] *p<0.05; **p<0.01; ***p<0.001

Abbreviations: CI = Confidence Interval, OR = Odds Ratio

(note: PBM abbreviated as "B")

Post-hoc analysis via estimated marginal means (EM means)

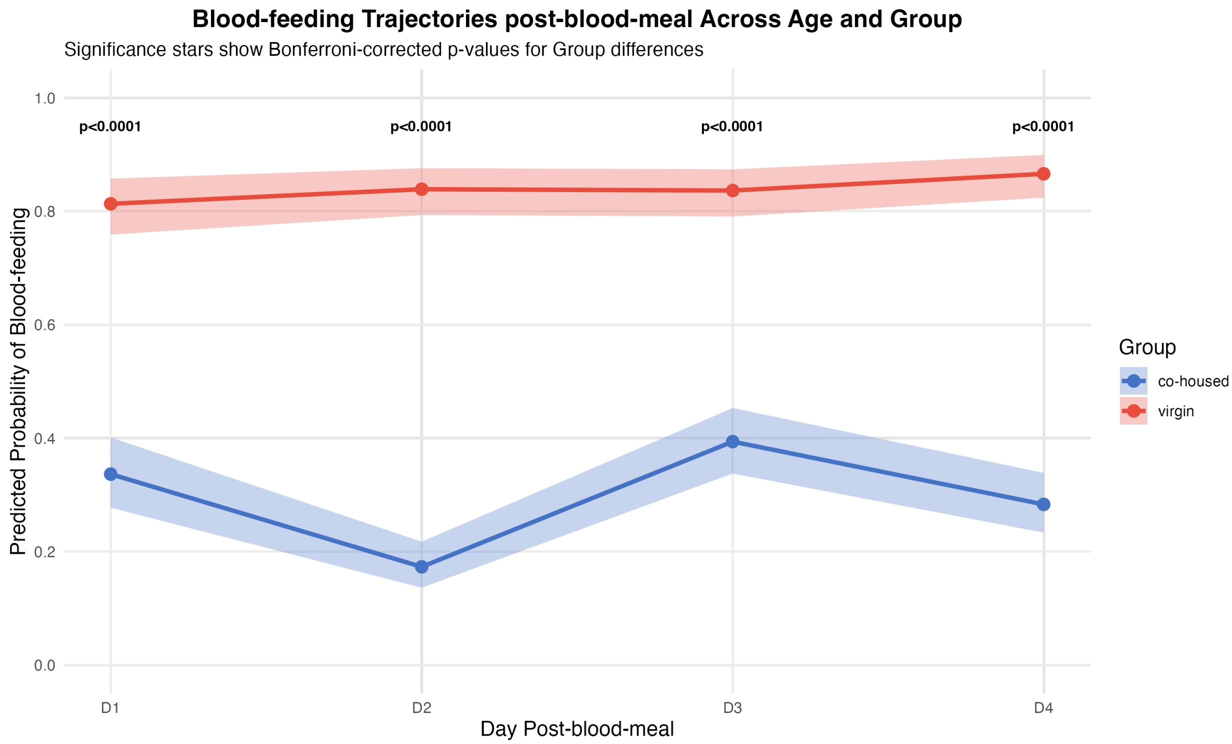
1) **Age-effects within each group (p-value adjustment: Tukey's method)**

```
Group = co-housed:
 contrast  estimate    SE  df z.ratio p.value
 DB1 - DB2   0.8849 0.188 Inf   4.703  <.0001
 DB1 - DB3  -0.2477 0.169 Inf  -1.467  0.4579
 DB1 - DB4   0.2499 0.175 Inf   1.424  0.4841
 DB2 - DB3  -1.1326 0.174 Inf  -6.498  <.0001
 DB2 - DB4  -0.6350 0.180 Inf  -3.524  0.0024
 DB3 - DB4   0.4976 0.161 Inf   3.092  0.0107

Group = virgin:
 contrast  estimate    SE  df z.ratio p.value
 DB1 - DB2  -0.1790 0.210 Inf  -0.852  0.8296
 DB1 - DB3  -0.1619 0.210 Inf  -0.773  0.8667
 DB1 - DB4  -0.3952 0.217 Inf  -1.822  0.2629
 DB2 - DB3   0.0171 0.202 Inf   0.085  0.9998
 DB2 - DB4  -0.2162 0.210 Inf  -1.032  0.7308
 DB3 - DB4  -0.2332 0.209 Inf  -1.117  0.6792

Results are given on the log odds ratio (not the response) scale.
P value adjustment: tukey method for comparing a family of 4 estimates
```

2) **Pair-wise comparison at each day (p-value adjustment: Bonferroni method)**

```
Day = DB1:
 contrast              estimate    SE  df z.ratio p.value
 (co-housed) - virgin     -2.15 0.199 Inf -10.800  <.0001

Day = DB2:
 contrast              estimate    SE  df z.ratio p.value
 (co-housed) - virgin     -3.21 0.199 Inf -16.129  <.0001

Day = DB3:
 contrast              estimate    SE  df z.ratio p.value
 (co-housed) - virgin     -2.06 0.180 Inf -11.492  <.0001

Day = DB4:
 contrast              estimate    SE  df z.ratio p.value
 (co-housed) - virgin     -2.79 0.194 Inf -14.397  <.0001

Results are given on the log odds ratio (not the response) scale.
```

(note: PBM abbreviated as "B")



<u>Conclusions:</u> Co-housed and virgin females show markedly different appetites, post-blood meal. Co-housed females (assumed mated), show a strong sustained suppression, while virgins continue to feed with high propensity, at least up to 4 days after a replete blood meal.

<u>Model 4: Whether the experimental groups differ in sugar-feeding behaviour across age?</u>

Random variables: BioID (replicate identity)

Fixed variables: Day(age), Group (co-housed vs virgin)

```
model4 <- glmer(Sugar.fed_binary ~ Day * Group + (1|BioID),
            data = mosquito_data_filtered,
            family = binomial)
```

Model summary:

Characteristic	Odds	Probability (%)	OR[1]	95% CI	p-value
Day					
D1	0.41	29.1	—	—	
D2	12.37	92.5	30.1***	15.6, 57.9	**<0.001**
D3	17.88	94.7	43.4***	20.6, 91.8	**<0.001**
D4	33.53	97.1	81.5***	31.6, 210	**<0.001**
D5	28.03	96.6	68.1***	28.3, 164	**<0.001**
Group					
co-housed	0.41	29.1	—	—	
virgin	0.88	46.8	2.13***	1.38, 3.27	**<0.001**
Day * Group					
D2 * virgin	0.21	17.4	0.51	0.21, 1.23	0.134
D3 * virgin	0.09	8.3	0.21***	0.09, 0.52	**<0.001**
D4 * virgin	0.16	13.8	0.39	0.12, 1.30	0.126
D5 * virgin	0.15	13.0	0.37	0.12, 1.13	0.080

[1] *p<0.05; **p<0.01; ***p<0.001

Abbreviations: CI = Confidence Interval, OR = Odds Ratio

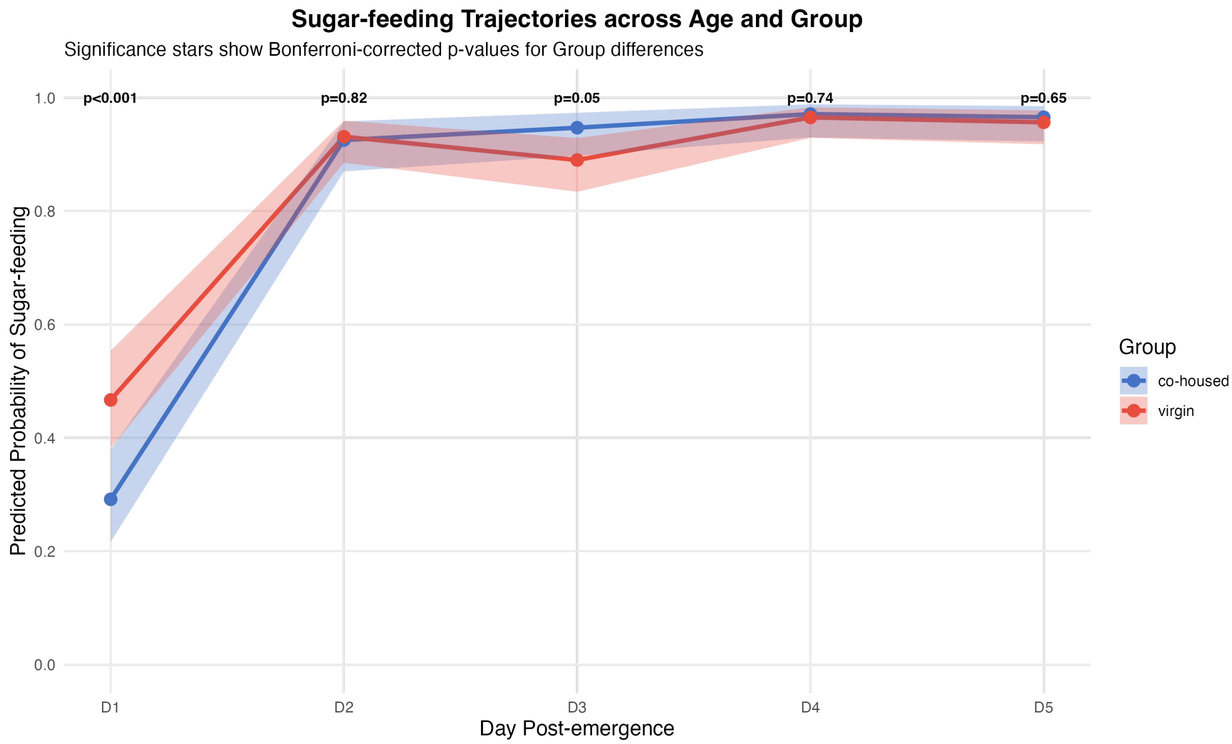
Post-hoc analysis via estimated marginal means (EM means)

1) Age-effects within each group (p-value adjustment: Tukey's method)

```
Group = co-housed:
 contrast estimate    SE  df z.ratio p.value
 D1 - D2    -3.403 0.334 Inf -10.185  <.0001
 D1 - D3    -3.771 0.382 Inf  -9.884  <.0001
 D1 - D4    -4.400 0.484 Inf  -9.093  <.0001
 D1 - D5    -4.221 0.448 Inf  -9.421  <.0001
 D2 - D3    -0.368 0.448 Inf  -0.823  0.9238
 D2 - D4    -0.997 0.537 Inf  -1.855  0.3419
 D2 - D5    -0.818 0.506 Inf  -1.617  0.4861
 D3 - D4    -0.629 0.568 Inf  -1.106  0.8032
 D3 - D5    -0.449 0.538 Inf  -0.835  0.9198
 D4 - D5     0.179 0.615 Inf   0.292  0.9984

Group = virgin:
 contrast estimate    SE  df z.ratio p.value
 D1 - D2    -2.737 0.296 Inf  -9.238  <.0001
 D1 - D3    -2.224 0.256 Inf  -8.685  <.0001
 D1 - D4    -3.454 0.387 Inf  -8.916  <.0001
 D1 - D5    -3.225 0.354 Inf  -9.114  <.0001
 D2 - D3     0.514 0.337 Inf   1.525  0.5461
 D2 - D4    -0.717 0.445 Inf  -1.613  0.4887
 D2 - D5    -0.488 0.416 Inf  -1.173  0.7668
 D3 - D4    -1.231 0.419 Inf  -2.937  0.0274
 D3 - D5    -1.001 0.388 Inf  -2.579  0.0743
 D4 - D5     0.230 0.485 Inf   0.474  0.9897

Results are given on the log odds ratio (not the response) scale.
P value adjustment: tukey method for comparing a family of 5 estimates
```

2) Pair-wise comparison at each day (p-value adjustment: Bonferroni method)

```
Day = D1:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin  -0.7546 0.219 Inf  -3.441  0.0006

Day = D2:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin  -0.0887 0.392 Inf  -0.226  0.8210

Day = D3:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin   0.7932 0.407 Inf   1.950  0.0512

Day = D4:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin   0.1911 0.582 Inf   0.328  0.7426

Day = D5:
 contrast             estimate    SE  df z.ratio p.value
 (co-housed) - virgin   0.2413 0.529 Inf   0.456  0.6485

Results are given on the log odds ratio (not the response) scale.
```



<u>Conclusions:</u> Both virgins and co-housed females show a sharp increase in their sugar-appetite from D1 to D2, followed by stabilization through D5.

Sugar-feeding behaviours are largely comparable between groups, across days. Virgin females demonstrated significantly higher initial feeding probability compared to co-housed females (D1). This difference was eliminated by D2 and remained non-significant thereafter.

Model 5: Effect of mating on the sugar-feeding behaviour of co-housed females

Random variables: BioID (replicate identity)

Fixed variables: Day(age), Mating status (yes or no)

```
model5 <- glmer(Sugar.fed_binary ~ Day * Mating + (1|BioID),
            data = mated_only,
            family = binomial)
```

Model summary:

Characteristic	Odds	Probability (%)	OR[1]	95% CI	p-value
Day					
D1	0.42	29.6	—	—	
D2	7.75	88.6	18.4***	8.19, 41.5	**<0.001**
D3	2.98	74.9	7.08***	2.64, 19.0	**<0.001**
D4	6.48	86.6	15.4***	4.32, 55.1	**<0.001**
D5	13511668.03	100.0	32,155,341	0.00, 458,391,919,179,031,447,577,326,110,191,003,156,070,149,023,901,469,250,371,113,818,366,346,793,494,511,616	0.841
Mating					
n			—	—	
y	1.70	63.0	4.05	0.92, 17.9	0.065
Day * Mating					
D2 * y	0.28	21.9	0.67	0.10, 4.44	0.682
D3 * y	1.76	63.8	4.18	0.52, 33.8	0.180
D4 * y	1.24	55.4	2.95	0.27, 31.7	0.373
D5 * y	0.00	0.0	0.00	0.00, 7,248,915,324,456,836,893,750,989,915,264,405,095,298,755,374,445,726,709,201,567,219,712	0.866

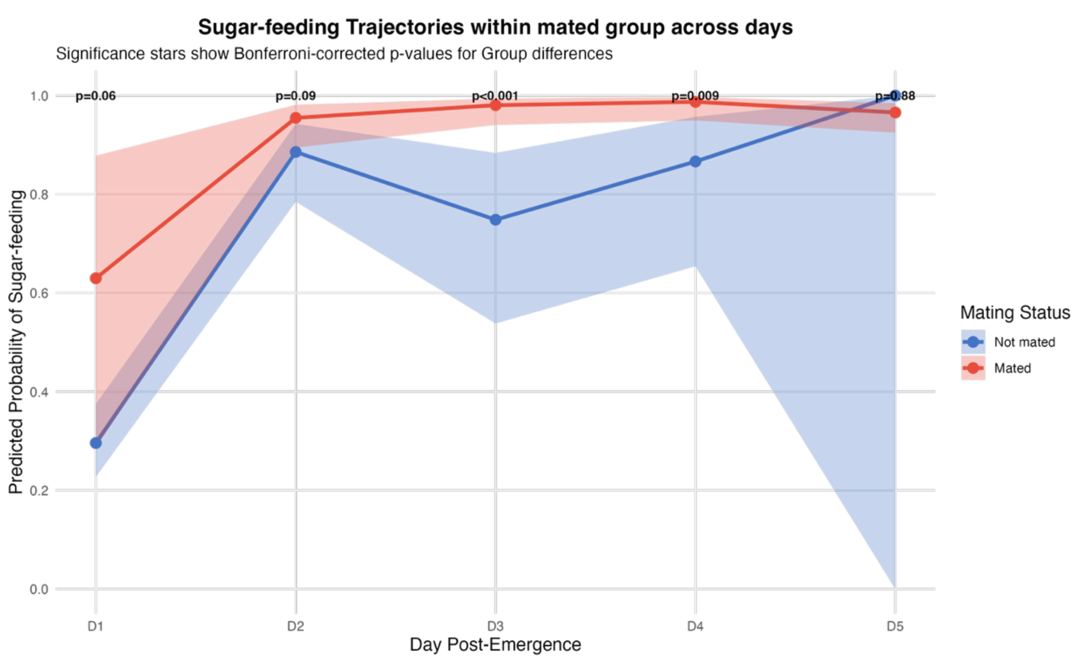
[1] *p<0.05; **p<0.01; ***p<0.001

Abbreviations: CI = Confidence Interval, OR = Odds Ratio

Post-hoc analysis via estimated marginal means (EM means)

Effect of mating (yes) on each day (p-value adjustment: Bonferroni method)

```
Day = D1:
 contrast estimate    SE  df z.ratio p.value
 y - n        1.40 0.757 Inf   1.846  0.0648

Day = D2:
 contrast estimate    SE  df z.ratio p.value
 y - n        1.00 0.596 Inf   1.684  0.0921

Day = D3:
 contrast estimate    SE  df z.ratio p.value
 y - n        2.83 0.754 Inf   3.753  0.0002

Day = D4:
 contrast estimate    SE  df z.ratio p.value
 y - n        2.48 0.946 Inf   2.620  0.0088

Day = D5:
 contrast estimate    SE  df z.ratio p.value
 y - n      -13.08 85.900 Inf  -0.152  0.8790

Results are given on the log odds ratio (not the response) scale.
```



Conclusions: Mating status shows only a brief, significant effect on sugar-feeding on D3 and D4 within co-housed females: mating positively influences sugar-feeding on these days. However, it is interesting to note that no differences were observed in the feeding behaviour of virgins and co-housed females (model 4) on these days. As mating sharply increases from D2 to D4, it possibly creates maximum differences on these days within the co-housed group, which are then cancelled out when averaged together.

Model 6: Whether the experimental groups differ in their sugar-feeding behaviour, post-blood meal, across age?

Random variables: BioID (replicate identity)

Fixed variables: Day(age), Group (co-housed vs virgin)

```
model6 <- glmer(Sugar.fed_binary ~ Day * Group + (1|BioID),
        data = mosquito_data,
        family = binomial)
```

Model summary:

Characteristic	Odds	Probability (%)	OR[1]	95% CI	p-value
Day					
DB1	0.02	2.0	—	—	
DB2	0.05	4.8	3.13	0.83, 11.8	0.093
DB3	10.11	91.0	631***	180, 2,219	**<0.001**
DB4	389103395.39	100.0	24,298,538,607	0.00, 347,082,619,877,364,307,044,218,276,151,296	0.358
Group					
co-housed	0.02	2.0	—	—	
virgin	0.22	18.0	13.6***	4.12, 45.2	**<0.001**
Day * Group					
DB2 * virgin	0.12	10.7	7.21**	1.74, 29.8	**0.006**
DB3 * virgin	0.01	1.0	0.43	0.08, 2.17	0.306
DB4 * virgin	0.00	0.0	0.00	0.00, 124,369,836,581,060	0.476

[1] *p<0.05; **p<0.01; ***p<0.001

Abbreviations: CI = Confidence Interval, OR = Odds Ratio

(note: PBM abbreviated as "B")

Post-hoc analysis via estimated marginal means (EM means)

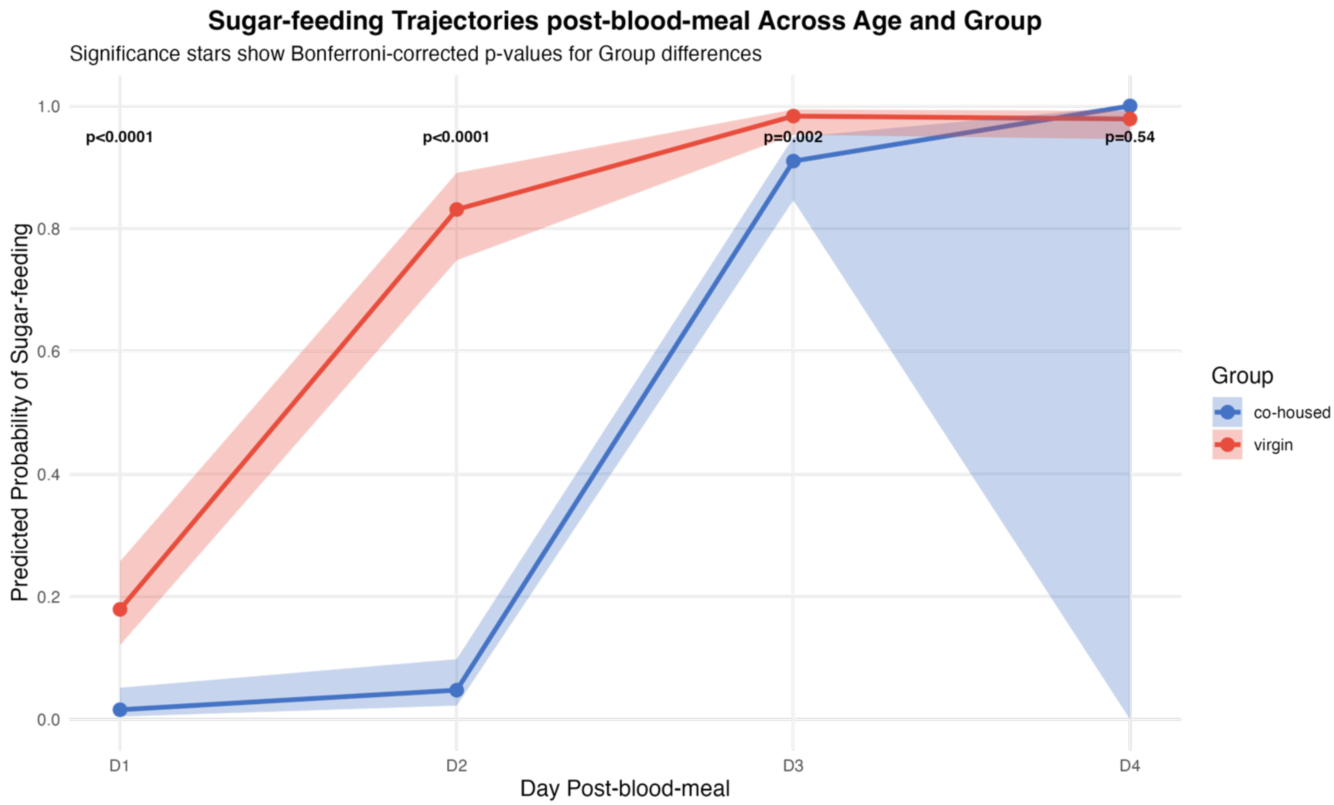
1) **Age-effects within each group (p-value adjustment: Tukey's method)**

```
Group = co-housed:
 contrast  estimate     SE  df z.ratio p.value
 DB1 - DB2    -1.14  0.678 Inf  -1.680  0.3343
 DB1 - DB3    -6.45  0.641 Inf -10.055  <.0001
 DB1 - DB4   -23.91 26.000 Inf  -0.919  0.7948
 DB2 - DB3    -5.31  0.433 Inf -12.246  <.0001
 DB2 - DB4   -22.77 26.000 Inf  -0.875  0.8179
 DB3 - DB4   -17.47 26.000 Inf  -0.671  0.9081

Group = virgin:
 contrast  estimate     SE  df z.ratio p.value
 DB1 - DB2    -3.12  0.257 Inf -12.141  <.0001
 DB1 - DB3    -5.60  0.539 Inf -10.387  <.0001
 DB1 - DB4    -5.35  0.491 Inf -10.896  <.0001
 DB2 - DB3    -2.49  0.538 Inf  -4.623  <.0001
 DB2 - DB4    -2.24  0.489 Inf  -4.569  <.0001
 DB3 - DB4     0.25  0.679 Inf   0.368  0.9830

Results are given on the log odds ratio (not the response) scale.
P value adjustment: tukey method for comparing a family of 4 estimates
```

2) **Pair-wise comparison at each day (p-value adjustment: Bonferroni method)**

```
Day = DB1:
 contrast             estimate     SE  df z.ratio p.value
 (co-housed) - virgin    -2.61  0.611 Inf  -4.275  <.0001

Day = DB2:
 contrast             estimate     SE  df z.ratio p.value
 (co-housed) - virgin    -4.59  0.398 Inf -11.523  <.0001

Day = DB3:
 contrast             estimate     SE  df z.ratio p.value
 (co-housed) - virgin    -1.77  0.562 Inf  -3.141  0.0017

Day = DB4:
 contrast             estimate     SE  df z.ratio p.value
 (co-housed) - virgin    15.95 26.000 Inf   0.613  0.5401

Results are given on the log odds ratio (not the response) scale.
```

(note: PBM abbreviated as "B")



Conclusions: Both virgins and co-housed females (assumed mated) show a repressed appetite for sugar immediately after a blood meal (D1 post-blood meal). This repression is significantly stronger in mated females. Virgins start to regain their appetite by D2 post-blood meal, while the mated females continue to suppress it. By D3 post-blood meal, the appetite is restored for both groups.