

# **Online Materials**

**APPENDIX**

In this appendix the procedure is explained that we used for the statistical analysis of the frequencies with which queens were present at a POP, and in which by one of the queens an egg was laid. This procedure was originally designed by Dr. Faber, formerly statistician at the Faculty of Biology, Utrecht University.

Consider the 1998-A colony in Table I. We want to know whether the observed frequencies for the three queens, in each of the four situations, differ from the frequencies that are to be expected under the null hypothesis that the presence of each queen at a POP is independent of the presence of the other queens. The observed proportion of queen B being present at a cell is:

$P_{(B)}$  = (number of times that B is present)/(total number of times that at least one of the queens is present) = 337/476. Similarly, for queens G and W:  $P_{(G)}$  = 260/476 and  $P_{(W)}$  = 198/476.

To facilitate the reading of the following calculations  $P_{(B)}$  is denoted by *b*,  $P_{(G)}$  by *g* and  $P_{(W)}$  by *w*. Under the null hypothesis of independence the expected frequencies with which queen B is present alone or together with one of the two other queens, or together with both other queens can be calculated as:

$$\begin{aligned} E(B \text{ alone}) &= b \times (1-g) \times (1-w) \times 476, \\ E(B \text{ and G present}) &= b \times g \times (1-w) \times 476, \\ E(B \text{ and W present}) &= b \times (1-g) \times w \times 476, \\ E(B, G \text{ and W present}) &= b \times g \times w \times 476. \end{aligned}$$

The expected frequencies for queens G and W are calculated similarly. The sum total of these expected frequencies (439.16) is less than 476, the value given in Table I. This is because in addition to the calculated probabilities for each of the queens to be present, our statistical model also incorporates the possibility that none of the queens is present. However, as a POP can only be started by a queen, it is inherently impossible to record this discrete event with no queen present. Nevertheless, we

can calculate the expected chance,

$$\begin{aligned} E(\text{none of the 3 queens present}) &= \\ (1-b) \times (1-g) \times (1-w) &= 0.077. \end{aligned}$$

Consequently the table as presented in Table I is statistically incomplete and should in fact contain one extra entry that is the “observed” number of times that none of the three queens were present. A way to solve this problem is to impute a value for the situation “none of the 3 queens present” such that this imputed value is exactly equal to what is to be expected under the null hypothesis of independence. The expected frequency for “none of the 3 queens present” can be calculated as:

$$\begin{aligned} E(\text{none of the 3 queens present}) &= \\ (1-b) \times (1-g) \times (1-w) \times T. \end{aligned}$$

Here T is the total number of events with 0, 1, 2 or 3 queens present at a cell. Note that T is not 476, but 476 + I, where I is the imputed number of times that none of the 3 queens were present. As a consequence of this imputation, the proportion with which each of the three queens is present is now calculated as:

$$\begin{aligned} P(B) &= 337/T, \\ P(G) &= 260/T, \\ P(W) &= 198/T. \end{aligned}$$

By means of an iterative procedure the imputed value I was found to be 69.54. The proportion *b* now equals 337/(476+69.54) = 337/545.54 = 0.62; similarly, *g* = 0.48 and *w* = 0.36. It can easily be checked that E(none of the 3 queens present) = 69.54 and thus exactly equal to the imputed value I, as was required. The table below presents the expected frequencies when the value 69.45 has been imputed for the situation “none of the 3 queens present”.

**EXPECTED FREQUENCIES**

Given these expected frequencies, the  $\chi^2$ -value turns out to be 3.92, which is not significant ( $P = 0.27$ , d.f. = 3). The following

Queen	Single	B+G	B+W	G+W	All 3 queens present	None of the 3 queens present	Total
B	112.37	102.32	64.02		58.29		337
G	63.32	102.32		36.07	58.29		260
W	39.62		64.02	36.07	58.29		198
Total	159.89	102.32	64.02	36.07	58.29	69.54	545.54

shows that there are three degrees of freedom. Calculation of the expected frequencies under the null hypothesis of independence uses the values of the observed frequencies with which each queen was present at a POP (337, 260 and 198 for B, G and W, respectively) and the total number of POPs (476) observed. Since there are 7 non-identical entries in the table (118, 58,

45, 102, 53, 36 and 64) the degrees of freedom equals 3.

In the situation with only two queens (colony 2000-II in Tab. I) there are no degrees of freedom left, because there are three non-identical entries (62, 105 and 93) in the table and three total counts (111, 82 and 156) that are used to calculate the expected frequencies.