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## SPECIALIA

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## Exact Test for Opposite Trends of Probability Changes in One or Several 3×2 Tables

In discrimination tasks in two-alternative choice experiments, the responses of a conditioned subject may sometimes be appropriately classified in more than two mutually exclusive categories. For example, it may be hard to constrain an animal in a choice experiment to a forced choice as is the usual practice in human subjects. From an ethologist's and a mathematical psychologist's point of view, it may be preferable to start with a division of the occurring overt behavioural responses in a certain number of recognizable behavioural elements. Each of the responses thus classified can then be paired with the stimulus type by which it was evoked.

As a special case we consider here the behaviour of a marine fish, the cod, in a food reward conditioning experiment in a fjord, in which the subject has to discriminate whether sound was incident from left or right of the netting cage in which it was kept 1. The stimulus could be emitted at random via one or the other of a pair of sound sources equidistant from the fish. In the list with the detailed descriptions of response types 1, a division in directional responses finally ending near the correct or the wrong food dispenser<sup>2</sup> at that particular trial, and non-directional responses, served to label 3 categories of responses (indicated by +, - and  $\times$ ). In control experiments the sound projectors were brought close together near the symmetry plane of the arrangement so that the sound 'image' was close to one or either side of that (imaginary) plane during a trial. As the observations show (Table), there are indications that the probability of non-directional responses increases in control experiments (7.3° inclination), and that the probability of correct responses decreases in such trials. How to detect such an effect in the training results of one or several individuals is the object of this paper. Pure tones of 75 Hz were used. The distance to the sources was about 4 m.

Observed frequencies of the responses

Subject		G3		G 5		G7	
Inclination		61	7.3	61	7.3	61	7.3
Response type	<del>-</del>	14 2	$\frac{1}{0}$	20 4	0 0	17 2	1 1
	×	6	3	1	4	0	1

Similar tables can be formed in acoustic lateralization experiments in human subjects, when 3 possible judgements are allowed: left, right and middle, which are then compared with the true stimulus situation. The detection theoretical model for this discrimination problem was given by Luce and Galanter3. Strictly speaking the legitimacy of joining the various responses to stimuli from sources left or right in common categories should be tested in advance.

The data are denoted in the following way (see Cono-VER4).

Let  $p_{ij}$  be the probability that the response falls in category i (i = 1, 2, 3) under condition j (j = 1, 2), then the nullhypothesis to be tested is

$$\begin{array}{l} {\rm H_0:} \; p_{i_1} = p_{i_2} \; (i=1,\,2,\,3) \; {\rm against \; the \; alternative \; hypothesis} \\ {\rm H_1:} \; p_{11} > p_{12} \; {\rm and} \; p_{31} < p_{32} \\ {\rm Further:} \; p_{1j} + p_{2j} + p_{3j} = 1, \quad j=1,\,2 \end{array} \tag{1} \end{array}$$

Further: 
$$p_{ij} + p_{gi} + p_{gi} = 1$$
,  $i = 1, 2$  (2)

When  $\vec{p}$  denotes the vector  $\vec{p} = (p_{11}, p_{21}, \dots, p_{32})$  then (2) constitute a 4-dimensional subspace  $\vec{p}$ .

Hence H<sub>0</sub> and H<sub>1</sub> may be rewritten respectively as:

$$\begin{split} & H_{0}: p \, \varepsilon \, \omega_{\, def} \, \big\{ \, p \mid p_{i_{1}} = p_{i_{2}}, \, i = 1, \, 2, \, 3 \, \big\} \\ & H_{1}: p \, \varepsilon \, \Omega - \omega_{\, def} \, \big\{ \, p \mid p_{11} > p_{12}, \, p_{31} < p_{32} \, \big\} \end{split} \tag{1a}$$

Let  $\hat{p}$  be the maximum likelihood estimate of  $\vec{p}$  under the assumption of  $H_0$  and  $\vec{p}^*$  be a similar estimate under  $H_1$ , then the test-statistic T of the likelihood ratio test is

$$T = \frac{\hat{p}_1}{p_{11}^*} \frac{0_{11} + 0_{12}}{p_{21}^*} \frac{\hat{p}_2}{p_{31}^*} \frac{0_{21} + 0_{22}}{p_{31}^*} \frac{\hat{p}_3}{p_{32}^*} \frac{0_{31} + 0_{32}}{p_{32}^*},$$
(3)

where 
$$p = (n_1/N, n_2/N, n_3/N)$$
. (4)

<sup>1</sup> A. Schuijf, submitted to J. comp. Physiol.

 $^{2}% \left( 1\right) =\left( 1\right) \left( 1\right)$ paper of M. A. VAN ARKEL, W. MAASSE and A. Schuijf, Experientia 29, 642 (1973).

<sup>3</sup> R. D. LUCE and E. GALANTER, in Handbook of Mathematical Psychology (R. D. Luce, R. R. Bush and E. Galanter; Wiley, New York 1963), vol. 1, p. 191-243.

<sup>4</sup> W. J. Conover, Practical Nonparametric Statistics (Wiley, New York 1971).

The following problems remain to be solved: 1. Computation of  $p^*$ . 2. Determination of the exact distributiod of T under  $H_0$  for fixed marginal totals  $n_1$ ,  $n_2$ ,  $n_3$ ,  $C_1$  ann  $C_2$  similar to procedures described by Conover<sup>5</sup>. 3. Determination of the P-value:  $P = Prob\{reject a true\}$  $H_0$ . 4. Combination of the test results obtained in experiments with different individuals.

ad 1. The random variable T assumes a discrete value for each possible  $3 \times 2$  table with given marginal totals. The cell frequencies in such a table are denoted by  $n_{ij}$ (i = 1, 2, 3; j = 1, 2) to distinguish them from the observed values Oij. The generation of the set of all these tables with a digital computer is simple. For each table the optimum value of  $\vec{p}^*$  is determined that maximizes the denominator of the statistic T with the current values of nij. In fact the optimization under H<sub>I</sub> is a constrained optimization: the solution is subject to the conditions found in (1a). a) the solution vector  $p^*$  of the unconstrained problem:

$$\overrightarrow{p}^* = (n_{11}/C_1, n_{21}/C_1, n_{31}/C_1, n_{21}/C_2, n_{22}/C_2, n_{32}/C_2)$$
(5)

satisfies provided that in (5)  $p_{11}^* \geq p_{21}^*$ , and  $p_{31}^* \leq p_{32}^*$ , which means  $n_{11}/C_1 \geq n_{12}/C_2$  and  $n_{31}/C_1 \leq n_{32}/C_2$ . b) if  $n_{11}/C_1 < n_{12}/C_2$  then put  $p_{11}^* = p_{12}^* = n_1/N$ . For the remaining subspace of p we find a solution  $p^*$  that differs from that in case d) (see below), provided that  $p_{31}^* \leq p_{32}^*$  is fulfilled. Then the maximum likelihood estimate is:  $p_{21}^* = n_{21} \, (N-n_1)/N(n_{21}+n_{31}), \, p_{31}^* = n_{31} \, (N-n_1)/N(n_{21}+n_{31}), \, p_{32}^* = n_{31} \, (N-n_1)/N(n_{21}+n_{31}), \, p_{32}^* = n_{32} \, (N-n_1)/N(n_{22}+n_{32}).$  It is concluded that the condition  $n_{32} \, (N - n_1)/N \, (n_{22} + n_{32}).$  It is continued that the condition for case b) is:  $n_{11}/C_1 < n_{12}/C_2$  and  $n_{31}/(n_{21} + n_{31}) \leq n_{32}/(n_{22} + n_{32}).$  c) if  $n_{31}/C_1 > n_{32}/C_2$  and  $n_{11}/(n_{11} + n_{21}) \geq n_{12}/(n_{12} + n_{22})$  then put  $p_{31}^* = p_{32}^* = n_3/N$ . Similarly as under b):  $p_{11}^* = n_{11} \, (N - n_3)/N \, (n_{11} + n_{21}),$   $p_{21}^* = n_{21} \, (N - n_3)/N \, (n_{11} + n_{21}),$   $p_{12}^* = n_{12} \, (N - n_3)/N \, (n_{12} + n_{22}),$   $p_{22}^* = n_{22} \, (N - n_3)/N \, (n_{12} + n_{22}).$  d) If none of the previously mentioned conditions is satisfied (those in b) and c) exclude each other) then put  $p_{i_1}^* = p_{i_2}^* = \hat{p_i}$ (i = 1, 2, 3); see (4).

An example with a conditional Table obtained from the data on subject G7 (Table), leading to the solution 1c, will illustrate the calculus.

18 0 | 18 
$$\hat{p}_1 = 18/22$$
  $p_{31}^* = p_{32}^* = \hat{p}_3$   
0 3 3  $\hat{p}_2 = 3/22$   $p_{21}^* = 0$   
1 0 1  $\hat{p}_3 = 1/22$   $p_{12}^* = 0$   
19 3 22

$$p_{11}^* = \frac{18 \times 21}{22 \times 18} = \frac{21}{22} \qquad p_{22}^* = \frac{3 \times 21}{22 \times 3} = \frac{21}{22}$$

$$T = \frac{\hat{p}_{11}^{19} \, \hat{p}_{2}^{3} \, \hat{p}_{3}}{p_{11}^{*3} \, \hat{p}_{3}} = \left(\frac{18}{21}\right)^{18} \left(\frac{3}{21}\right)^{8} = 1.82 \times 10^{-4}$$

note: put  $0^0 = 1$ 

ad 2. Assume that the generated sequence of conditional tables  $[n_{ij}]$  contains m tables. Assign a key number  $h = 1, 2, \ldots, m$  to each table in order to sort<sup>7</sup> the sequence of associated values of the statistic T as to increasing values. The identity of all the possible events (tables) connected to the T outcomes in the sorted sequence is preserved in a separate array of the key numbers, in which the elements get the same permutations as in the sorted array of T values. The correspondence of each table with its sorted T-value is thus solved. The conditional probability that some table  $[n_{ij}]$  occurs under H<sub>0</sub> equals<sup>5</sup>:

prob. = 
$$\binom{n_1}{n_{11}} \binom{n_2}{n_{21}} \binom{n_3}{n_{31}} \binom{N}{C_1}^{-1}$$
 (6)

An Algol 60 algorithm for computing the required binomial coefficients is found in reference8.

For the table chosen in the previous example the rank r = 1, and the probability  $\pi_1$  that this event occurs

$$\pi_1 = \binom{18}{18} \binom{3}{0} \binom{1}{1} \binom{22}{19}^{-1} = 6.49 \times 10^{-4}.$$

ad 3. The P-value of the observed table  $[O_{ij}]$  is calculated by adding the probabilities of all the tables that satisfy  $T \leq T_{\rm observed}$ . From the data of the Table the following values of P were computed  $^9$ :  $P_1=0.122$  (G3),  $P_2=2.53\times 10^{-4}$  (G5) and  $P_3=3.77\times 10^{-2}$  (G7). From now on the index i will be used to indicate the independent experiments.

ad 4. The k one-sided tests are combined with Fisher's omnibus procedure. The examined case of the three  $3 \times 2$ tables is further exemplified (k = 3). The hypothesis  $H_0$ is rejected for small values of the product test statistic Q.

$$Q = \prod_{i=1}^{k} = P_1 P_2 P_3$$

in which the p-value of the test results in the different experiments are considered as independent random variables (in bold type) denoted by  $P_i$ . The distribution of  $P_i$  has  $m_i$  discrete probabilities  $\pi_{ir}$  (i = 1, 2, ..., k;  $r = 1, 2, ..., m_i$ ), that were calculated before by means of (6). Wallis 10 has studied the computation of exact tail probabilities (P-values) of the Q statistic for discrete  $P_i$ (i = 1, ..., k). The sample space 4 consists of  $m_1 m_2 m_3 =$  $12 \times 15 \times 7$  points: all the outcomes of

$$\sum_{r=1}^{a} \pi_{\hat{1}r} \sum_{r=1}^{b} \pi_{\hat{2}r} \sum_{r=1}^{c} \pi_{\hat{3}r} \ (a=1,...,m_1; \ ; c=1,....,m_3).$$

The associated probabilities are  $\pi_{1a}\pi_{2b}\pi_{3c}$ . The outcomes of **Q** are ranked with the same procedure 7 as before.

The result of the combination problem is P = Prob $\{Q \le P_1 P_2 P_3\} = \text{Prob}(Q \le 1.165 \times 10^{-6}) = 1.56 \times 10^{-5}.$ If a continuous distribution had been assumed for the  $P_i$ , the results would have been 11  $(Q = P_1 P_2 P_3)$ 

$$P = Q + Q \sum_{i=1}^{k-1} (-\ln Q)^{i} /_{i}! = 1.26 \times 10^{-4}.$$

- <sup>5</sup> W. J. Conover, Practical Nonparametric Statistics (Wiley, New York 1971), chapter 4.
- <sup>6</sup> The author is greatly indebted to Dr. J. Oosterhoff, Mathematical Institute, University of Nijmegen, The Netherlands, for introducing the subclasses 1b and 1c in the computation of vector  $p^*$  and for the explicit expressions in these cases which eliminated the need for a numerical optimization. Further more valuable remarks regarding the combination problem were obtained.
- <sup>7</sup> T. N. Hibbard, Communs, Ass. Comput. Mach. 6, 206 (1963),
- 8 Communs, Ass. Comput. Mach., Collected algorithms Nr. 19-P1-0.
- <sup>9</sup> Print outs of the Algol 60 program for the test examined here are available on request addressed to Ir. A. Schuijf.
- W. A. Wallis, Econometrica 10, 229 (1942).
  K. Pearson, Biometrika 25, 379 (1933).

In the paper on directional hearing in cod<sup>1</sup>, it was therefore concluded that the direction of the sound sources is decisive for the discrimination and not the timbre or intensity differences between the sound sources.

Zusammentassung. Der «Likelihood»-Quotiententest wird auf einer 3×2-Kontigenztafel mit unbekannten Wahrscheinlichkeiten  $p_{ij}$  (i = 1, 2, 3; j = 1, 2) angewandt, um die Hypothese  $H_0: p_{i1} = p_{i2}$  (i = 1, 2, 3) gegen die alternative Hypothese  $H_1: p_{11} > p_{12}, p_{31} < p_{32}$  zu prüfen, und zwar wenn kleine Stichproben vorhanden sind. Ausserdem wird die Kombination solcher Tests behandelt. Als Beispiel wird diese Testtheorie beim Wahlverhalten des Kabeljaus im Dressurversuch über akustische Lokalisation in zwei Stimulussituationen verwendet.

A. Schuiff 6, 12

Laboratory of Comparative Physiology Jan van Galenstraat 40, Utrecht (The Netherlands), 17 December 1973.

<sup>12</sup> The close cooperation with Drs. E. Meelis, Institute of Theoretical Biology Leyden, The Netherlands, in applying the likelihood ratio test to this case is gratefully acknowledged.

## The 2,2'-Dicyano-1,1'-Dimethyl-4,4'-Bipyridylium Di-cation: A Viologen Indicator with a High Redox Potential

Diquaternary salts of 4,4'-bipyridyl, especially methyl viologen (I; R = CH<sub>3</sub>) and benzyl viologen (I; R = CH<sub>2</sub>.Ph)<sup>1</sup> have been used considerably as redox indicators and electron carriers in biological systems<sup>2</sup> and in chemical reactions3. These salts are reduced in aqueous solution by a one electron transfer which is reversible to give highly coloured stable radical cations (e.g. II). The salts originally studied  $^1$  had redox potentials (E<sub>o</sub>) between -0.36and -0.45 volts. With the discovery of the phytotoxic properties of (I; R = CH<sub>3</sub>)<sup>4</sup> and its development as the herbicide paraquat interest in salts of this type has increased. Several diquaternary salts of 2,2'-bipyridyl, for example, the herbicide diquat (III)4, behave similarly as redox systems and they too have found applications as electron carriers and indicators in biological 5-9 and chemical 10 systems. Reversible redox indicators based on diquaternary salts of 2, 2'- and 4, 4'-bipyridyls have thus been extended over a range from about -0.15 to -0.70volts<sup>4,7-9,11-17</sup>. We now report the preparation of a viologen indicator with a much higher redox potential.

4,4'-Bipyridyl-1,1'-dioxide $^{18}$  (1.0 g) and dimethyl sulphate (5 ml) were heated at 150°C for 30 min. The cooled solution was added to ethyl acetate (100 ml) and the resultant white precipitate of 1,1'-dimethoxy-4,4'-bipyridylium dimethosulphate was collected (cf. ref. 18). Without purification the salt was dissolved in water (20 ml) and the pH adjusted to 10.0 with sodium carbonate solution. Potassium cyanide (2 g) in water (15 ml) was added and a white precipitate of 2,2'-dicyano-4,4'-bipyridyl (IV) formed immediately (cf. ref. 19).

- L. MICHAELIS and E. S. HILL, J. gen. Physiol. 16, 859 (1933).
   For example: H. Gest and H. D. Peck, J. Bact. 70, 326 (1955). J. C. SADANA and W. D. McElroy, Arch. Biochem. Biophys., 67, 16 (1957). - R. C. VALENTINE, L. E. MORTENSON, H. F. MOWER, R. L. JACKSON and R. S. WOLFE, J. biol. Chem. 238, 857 (1963). -M. S. NAIK and D. J. D. NICHOLAS, Biochim. biophys. Acta 131, 204 (1967). - R. H. GARRETT and A. NASON, J. biol. Chem. 244, 2870 (1969). - H. Strotmann and C. von Goesseln, Z. Naturforsch. 27b, 445 (1972). - D. R. BIGGINS and J. R. POSTGATE, Eur. J. Biochem. 19, 408 (1971).
- <sup>3</sup> For example: N. K. King and M. E. Winfield, J. Am. chem. Soc. 80, 2060 (1958). - A. S. Hopkins, A. Ledwith and M. F. STAM, J. chem. Soc. D., 1970, 494.
- 4 R. F. HOMER, G. C. MEES and T. E. TOMLINSON, J. Sci. Fd. Agric. 11, 309 (1960).
- <sup>5</sup> C. C. Black, Science 149, 62 (1965).
- <sup>6</sup> C. W. M. ORR, Biochem. J. 100, 20 P (1966).
- <sup>7</sup> G. Zweig and M. Avron, Biochem. biophys. Res. Commun. 19, 397 (1965).
- <sup>8</sup> B. Kok, H. J. Rurainski and O. V. H. Owens, Biochem. biophys. Acta 109, 347 (1965).
- <sup>9</sup> C. C. Black, Biochem. biophys. Acta 120, 332 (1966).
- 10 For example: R. Arnold and D. A. Swift, Austr. J. Chem. 22, 859
- <sup>11</sup> J. E. Downes, J. chem. Soc. C., 1967, 1491.
- <sup>12</sup> S. Huenig, J. Gross and W. Schenk, Liebigs Annln. Chem. 1973,
- <sup>13</sup> B. G. White, Proc. 10th Brit. Weed Control Conf. 1970, p. 997.
- <sup>14</sup> J. E. Dickeson and L. A. Summers, Experientia 25, 1247 (1969).
- <sup>15</sup> J. E. Dickeson and L. A. Summers, J. heterocyclic Chem. 7, 401 (1970).
- <sup>16</sup> A. L. Black and L. A. Summers, J. heterocycl. Chem. 8, 29 (1971).
- $^{\rm 17}$  A. L. Black and L. A. Summers, J. chem. Soc. C., 1971, 2271.
- <sup>18</sup> R. Fielden and L. A. Summers, J. heterocycl. Chem., in press. <sup>19</sup> E. Ochiai, Aromatic Amine Oxides (Elsevier, New York 1967),