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Some Models Describing the Distribution of Eggs of the Parasite *Pseudeucoila bochei* (Hym., Cynip.) over Its Hosts, Larvae of *Drosophila melanogaster*

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Summary. Ovipositing females of the cynipid wasp *Pseudeucoila bochei* discriminate between parasitized and unparasitized hosts, which results in a far more uniform distribution of eggs over the hosts than would be obtained if oviposition were random (Fig. 1, a_0–f_0).

For the description of the distributions a few models were worked out, which rest on the assumption that the hosts are probed at random. The total number of effective probes made in a larva during the experiment is a random variable with a Poisson distribution and an expectation λ. The chance that at a certain probe an egg will be laid (δ) is dependent on the number of eggs present (j); $1=\delta_0>\delta_1\geqq\delta_2\geqq\delta_3\ldots$.

In model I it was assumed that the female had only the ability to distinguish parasitized from unparasitized hosts. The chance that an egg will be laid in an unparasitized host when it is probed, δ_0, is considered to be equal to 1, while $\delta_1=\delta_2=\cdots=\delta_n<1$ (Fig. 2, 1, a_1—f_1). When the mean number of eggs present in a host was larger than about 1.1, this model did not describe the distributions of eggs satisfactorily (Fig. 3).

It seemed that the ovipositing female is not only able to distinguish parasitized from unparasitized hosts, but also to distinguish the *number* of eggs present in a host. In model II it was assumed that the wasp could distinguish between hosts with 0, 1, and 2 or more eggs: the chance that an egg would be laid in a host containing 2, 3, 4, . . . eggs was, hence, the same in this model $\delta_1<\delta_2=\delta_3=\cdots=\delta_n$ (Fig. 2, 1, c_2, e_2, f_2). This model described the distributions of eggs much better (Figs. 4 and 5), but at mean numbers of eggs per host above 2 it was apparently inadequate.

Two other models were then tried, in which the chance δ that an egg would be laid in a host decreased with the number of eggs already present (j). In model III (Fig. 2) the chance decreased according to the function $\delta_j=\delta/j$ ($\delta_0=1$, $\delta<1$). Fig. 1, d_3, e_3, f_3, gives some examples. In model IV the chance $\delta_j=\delta^j$ ($\delta_0=1$, $\delta<1$) (see Fig. 1, d_4, e_4, f_4).

From the comparison of Figs. 6 and 7 it is clear that model IV gives the best description of the distributions of eggs found.

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The value of these models is discussed, and plans for both an approach through experimental analysis and simulation models are given. In an Appendix the mathematical derivation of the models is presented.

I. Introduction

The different aspects of the interactions between parasitic wasps and their hosts form ideal objects for interdisciplinary studies. One of the wasps chosen for such a study at our laboratory is the cynipid *Pseudeucoila bochei* Weld.

The morphology and general biology of the species have been studied extensively by Jenni (1951) and Nöstvik (1954). Meyer-Grassmann (1967) investigated the problem of elimination of supernumerary parasite larvae in a host. Much work has been done on the defence reactions of the host against the parasite, and on the ability to break down this defence reaction by certain parasite strains (Schlegel-Oprecht, 1953; Walker, 1959; Hadorn and Walker, 1960; Walker, 1961; Hadorn and Grassmann, 1962; Walker, 1962; Streams, 1968; Streams and Greenberg, 1969; Nappi and Streams, 1969, 1970).

At our laboratory work has been done on olfactory orientation related to host searching behaviour, host selection (Eijsackers and Van Lenteren, 1970; Bakker, 1971), oviposition behaviour, discrimination between parasitized and non-parasitized hosts (Bakker *et al.*, 1967), elimination of supernumerary parasite larvae (Eijsackers and Bakker, 1971), duration of development of the sexes (Eijsackers and Bakker, 1971) and courtship behaviour (Van den Assem, 1969).

The present study is an extension of the work on host discrimination published earlier (Bakker *et al.*, 1967).

The female of *Pseudeucoila bochei* lays her eggs in larvae of different species of *Drosophila*. When a female has found a place where host larvae are likely to be found, *e.g.* a substratum covered by a suspension of yeast, she extends her ovipositor a little and walks over the surface "pricking" her ovipositor rhythmically into the substratum, while moving her antennae up and down continuously, nearly touching the substratum. Exact localization of a host takes place by means of the ovipositor: when it "hits" a host it may "pierce" the host's skin and stay in this position for a few seconds. This component of behaviour we have called "probing". It is difficult to see whether the ovipositor only touches the host or actually pierces it, except when "probing" follows. It may lead to "egg-laying", in which case the wasp extends her ovipositor further and stays in this position for some time, generally about (15) 20–30 (40) seconds. Her abdomen makes quivering movements and her antennae move slowly up and down with a greater amplitude than when "pricking". After this she retracts her ovipositor, and, often after spending a short time preening, she walks away, again pricking her ovipositor rhythmically into the substratum. There are some indications that the wasp injects some paralyzing poison into the host larva before egg-laying, since the host which first may wriggle and try to crawl away after being pierced, becomes motionless soon afterwards and persists in that stage for about a minute. (Larvae that had been parasitized do not react to pricks delivered by a thin needle for about a minute after parasitization.)

Dissections of larvae that had been handled by the parasite as described above showed that, apart from certain special cases not to be discussed here in which no egg is found, one egg had always been laid at a time. The parasitized hosts develop further and form puparia from which the wasps emerge after about 2 weeks at 25° C.

One host produces only one parasite, hence there must be some mechanism that prevents superparasitization, or that eliminates supernumerary parasite larvae within one host when more than one egg is laid, or both. When the ratio of parasites to hosts is high, or when the hosts are subjected to the parasites for a longer time, two or even more eggs are often found in one host, which called for an explanation of the elimination of all but one parasite in such cases. It was shown (Meyer-Grassmann, 1967; Eijsackers and Bakker, 1971) that *elimination* of supernumerary larvae by physical attack of rivals does take place, though it is by no means certain that it is the only mechanism responsible.

In an earlier paper (Bakker *et al.*, 1967) it has been shown that the egg-laying female wasp does also *discriminate* between unparasitized and already parasitized hosts, but that this discrimination is not absolute and depends strongly on the availability of unparasitized hosts. A mathematical model was worked out in order to have some measure of the degree of discrimination under different conditions. In this model it was assumed that the wasp could discriminate between parasitized and unparasitized hosts, but that it was not able to distinguish whether only one, or more eggs were already laid in the host found. From some observations of the distribution of eggs laid under conditions where the mean number of eggs per host was higher than 1, we got the impression that this assumption was wrong. When a female encounters a host containing only one parasite egg the chance that she will lay another egg seems to be greater than when she meets a host in which already 2 eggs have been laid, and so on.

The present study has been made to investigate the problem of whether the female wasps are able to distinguish the number of eggs present in a host, and to develop a model describing the distribution of the eggs over the hosts that results from this ability.

II. Material and Methods

For rearing methods of flies and wasps the reader is referred to Bakker (1961) and Bakker *et al.* (1967). The parasites used in the majority of the experiments belonged to the strain "Leiden". For some preliminary experiments we used the strains "Brissago" and "Storrs", which we obtained from Mr. C. Singeisen (ETH, Zürich) and Prof. Dr. F. A. Streams (Storrs). In all experiments of sets I, II and III newly hatched larvae of *Drosophila melanogaster* were transferred to glass jars (4 cm diameter in sets I and II) or large petri dishes (20 cm diameter for set III) containing an agar base provided with a layer of suspended yeast and placed in an incubator at 25° C. When the larvae were 24 hours old, a varying number of female wasps was introduced for a varying period to obtain different degrees of parasitization. Since the eggs of the parasite are difficult to see immediately after they have been laid, because they are then entirely hyaline, the parasitized hosts were kept for about 20–24 hours at 25° C. During this period the parasite eggs developed and became more opaque, which made them easier to be found. The hosts were then washed from the medium through a fine nylon gauze, dissected immediately, or killed with warm water (about 60° C), and stored away in a deep freezer until they could be dissected. For dissection, they were placed in a drop of water on a black glass background. A magnification of 25 × and strong illumination appeared satisfactory for discovering the eggs of the parasite.

Set I consisted of 89 batches of about 50 hosts, randomly divided into two subsets, Ia of 47 and Ib of 42 batches. In set II 26 batches of 80–150 hosts were used (average 110), while set III contained 4 batches with 666, 750, 771, and 731 hosts (Table 1).

In total, about 10000 hosts have been dissected.

In experiments during which the parasites were continuously observed, the hosts were offered in small open petri dishes (5 cm diameter). It appeared that a female wasp actively engaged in searching for hosts and egg-laying does not escape from these dishes and that hosts that were apparently parasitized could be easily removed without disturbing the wasp.

Table 1. Number of batches of the different sets on which the different models were tried

	Model			
	I	II	III	IV
Set Ia				
$\bar{x} \geqq 0.50$	47	26	—	—
$\bar{x} \geqq 1.35$	—	—	21	21
Set Ib				
$\bar{x} \geqq 0.50$	—	—	42	—
$\bar{x} \geqq 1.30$	22	21	—	22
Set II				
$\bar{x} \geqq 1.10$	23	24	26	26
Set III	—	—	4	4

III. Preliminary Experiments and Discussion on Methods

When an unparasitized host has been hit by the ovipositor, egg laying does not always follow. We measured the number of times unparasitized larvae were hit, by scoring the number of touches with the ovipositor evoking a movement of the host, and calculated the ratio of "hits" to "ovipositions". This ratio is about 2 in most cases (average 1.9). Table 2 gives the separate figures for a number of individual females of different strains of parasites.

Table 2. Number of hits and probes to unparasitized hosts and number of eggs laid

Strain	Female no.	No. of "hits"	No. of "probes"	No. of eggs laid	Ratio hits/eggs	Ratio probes/eggs
Leiden	1	39	30	22	1.8	1.4
	2	50	37	31	1.6	1.2
	3	46	34	22	2.1	1.5
	4	52	30	25	2.1	1.2
Brissago	1	81	40	32	2.5	1.3
	2	48	39	33	1.5	1.2
	3	52	36	29	1.8	1.2
Storrs	1	60	46	34	1.8	1.4
	2	43	28	20	2.2	1.4
	3	47	36	28	1.7	1.3

However, in these experiments a "hit" has been recorded only on the basis of the behaviour of the host, not on that of the parasite. All cases in which the host reacted to the parasite, whether "probing" followed or not, have been taken together as "hits". The ratio of "probes" to "ovipositions" (average 1.3) is, of course, lower than that of "hits" to ovipositions, as is shown in Table 2.

A preliminary experiment was set up to investigate whether discrimination between parasitized and unparasitized hosts depends on contact with the ovipositor. For this purpose, three batches of 24 hour old hosts, consisting each of 25 unparasitized larvae and 25 larvae that were observed to have been parasitized (*i. e.* the wasp had inserted her ovipositor in these larvae for 15 seconds or longer) about 4 hours earlier, were presented to three individual fertilized female wasps (Storrs). The behaviour of these wasps towards both groups of host larvae was observed. (It was of course necessary to distinguish both groups, and this was achieved by placing one group before the experiment on yeast coloured with carmine, which remains clearly visible in the gut for a number of hours, or to colour one group externally by placing them in a drop of a saturated solution of Sudan-black in propylene glycol for five seconds. Colouring does not influence the host's chance of being hit or "parasitized", as is clear from Table 3.) The number of probes was recorded and every time a larva was seen to be parasitized, it was taken away, stored individually, and replaced by another larva of the same category (*i. e.* unparasitized or parasitized, uncoloured or coloured). The hosts "parasitized" during the experiment were dissected, as well as a control group of the hosts which were assumed to be "parasitized" before the experiment.

Table 3. Number of "hits" and "ovipositions" in coloured and uncoloured larvae. In each of the four experiments 40 coloured and 40 uncoloured larvae were presented to a female wasp

	No. of "hits"	
	Carmine	Sudan-black
Coloured	47	23
Uncoloured	53	25

	No. of "ovipositions"	
	Carmine	Sudan-black
Coloured	21	13
Uncoloured	19	14

The results are shown in Table 4. Both categories of larvae appear to be "probed", and, apart from female 3, to a similar degree. The third female "probed" more than twice as often in the parasitized larvae. This was due to the circumstance that she stayed for a long time in a particular part of the petri dish where about 7 "parasitized" larvae were aggregated, which she "probed" frequently. Only in a few cases "ovipositions" were observed in hosts assumed to be "parasitized" before the experiment. In these larvae, only one egg was always found. This may mean that these larvae were erroneously considered to be parasitized before the experiment or that at the second "oviposition", observed during the experiment, no egg was laid. The dissections of the controls showed that in some cases larvae have indeed erroneously been considered as being "parasitized" before the experiment.

Table 4. Number of probes and ovipositions by 3 individual parasites in 25 unparasitized hosts and in 25 hosts that were observed to have been parasitized before. Number of eggs found. After every "oviposition" the host was removed and replaced by a host of the same category

Female no.	Hosts	Coloured group	"Probes" observed	"Ovipositions" observed	Eggs found (1 per host)
1	Unparasitized	—	25	19	19
	"Parasitized"	+	26	2	2
	Control "parasitized"	+		46	43
2	Unparasitized	+	11	11	10
	"Parasitized"	—	10	4	4
	Control "parasitized"	—		41	40
3	Unparasitized	—	36	25	25
	"Parasitized"	+	77	1	1
	Control "parsitized"	+		24	24

We never found more than one parasite egg per larva in this experiment. It may be concluded that, in order to discriminate between parasitized and unparasitized hosts, the parasite has to use its ovipositor. Further, it appears that discrimination is rather strong.

In dissecting it is not unlikely that some eggs are overlooked. This could seriously affect the conclusions about the distribution of the eggs if the chance to overlook an egg is higher when more eggs have been laid in one host. Since all of us who have been engaged in dissecting were strongly aware of this possible error, we do not think it has played a significant role.

Another point is the fact that when the hosts were washed from their medium, often a number of them proved to be dead, and some of these could not be dissected anymore because they were already putrefied. Also, sometimes a few hosts were not retrieved, and therefore assumed to

Table 5. Average number of eggs found in living and dead hosts

No.	Total number of hosts	Living hosts		Dead hosts	
		Number	Average no. of eggs	Number	Average no. of eggs
1	89	62	1.74	27	1.67
2	156	136	1.82	20	1.65
3	151	130	2.08	21	1.95
4	155	137	2.44	18	2.11
5	118	106	1.85	12	1.83
6	128	97	1.64	31	1.84

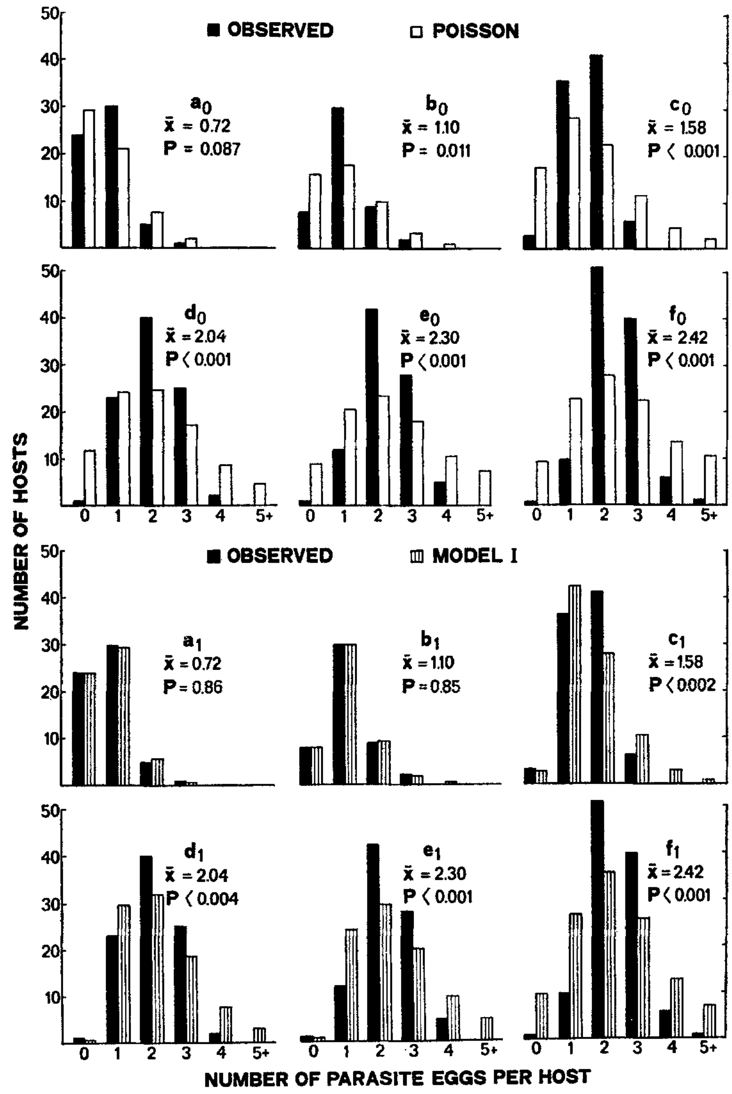

Fig. 1a

Fig. 1a and b. Frequency distributions of hosts containing 0, 1, 2, 3, 4 or ≥ 5 parasite eggs. Examples *a*—*f* with increasing mean numbers of eggs per host ($\bar{x}$). a_0–f_0: comparison of distributions observed with Poisson distributions with the same mean. a_1—f_1: comparison of distributions observed with those obtained with model I. c_2, d_2, f_2: the same for model II. d_3, e_3, f_3: the same for model III. d_4, e_4, f_4: the same for model IV

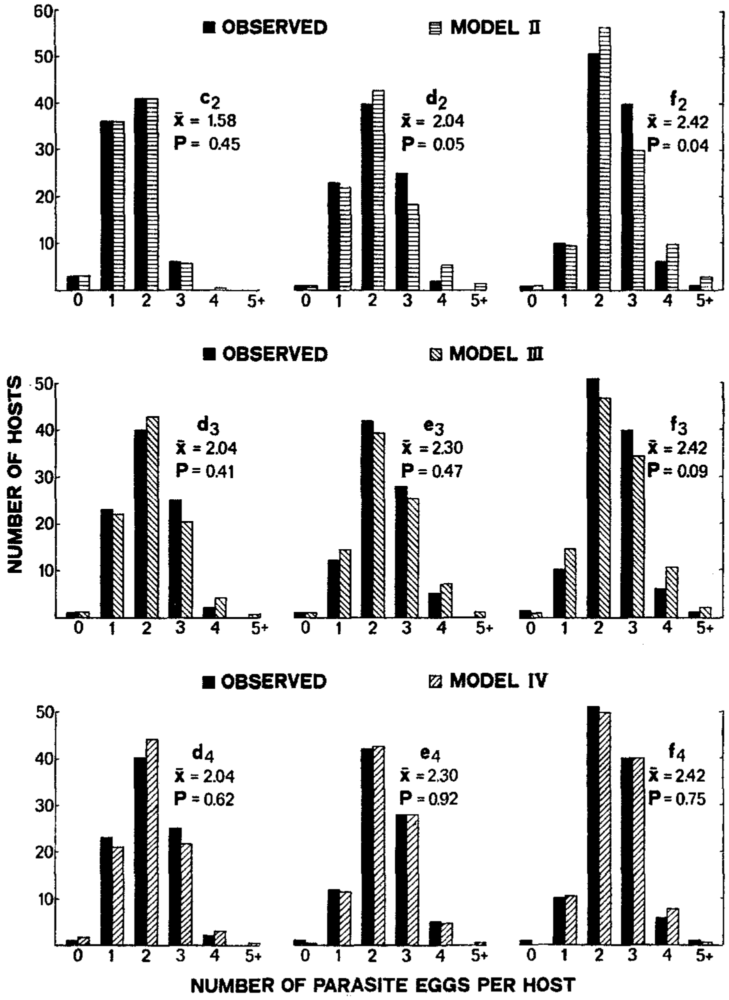

Fig. 1b

be dead and putrefied. Now it could be possible that those larvae that died early in the experiment and could not be dissected, or were not retrieved, were those containing on the average a larger number of eggs than those that stayed alive or died only later in the experiment and were dissected. The average number of eggs in living larvae is, however, very similar to that in the dead larvae that could be dissected as shown in Table 5. The differences were very small, and, if anything, the mean number of eggs in dead larvae appeared to be slightly smaller, not larger, than that in living larvae. We therefore felt justified to consider the figures of the distribution of eggs over the larvae that were dissected as representative for the distribution of the eggs over all hosts that had been present.

IV. Results and Description of the Models

Some of the distributions obtained are pictured in Fig. 1, a_0–f_0, and compared with Poisson distributions with the same mean (see also Table 6). It is clear that the distributions observed are much more regular, *i. e.* the variance is much smaller than expected. Apparently the Poisson distribution does not describe the distribution of the parasite eggs over the hosts properly: at lower mean numbers of eggs per host ($\bar{x}$) the number of hosts containing no eggs at all is far lower than expected, while the number of hosts with one egg is much higher than expected. At higher mean numbers of eggs per host also the number of hosts with one egg becomes lower than expected, while the number of hosts with 2 and 3 eggs becomes clearly larger.

The 4 mathematical models, which were developed for the description of the distributions observed, are all derived from the following general model.

During each experiment with a certain number of larvae (n) each host larva (i) is probed Z'_i times. It is assumed that Z'_i is a random variable which has a Poisson distribution with an unknown expectation μ; $i=1, \ldots, n$.

Further it is assumed that $Z'_1, \ldots, Z'_n$ are mutually independent. After each probe, the searching female wasp makes her next probe at random to one of the n hosts present.

As we have seen in section II, there is a ratio of probes to eggs of about 1.3 (Table 2). Therefore we introduce the concept of *effective probe.* We assume that from the total number of probes only a certain fraction p is effective in the sense that it is possible that the probe results in an oviposition.

As is shown in the Appendix, part A, the total number of effective probes into a larva (i) during the experiment is a random variable Z_i, $i=1, \ldots, n$, with a Poisson distribution and an expectation λ which is equal to p. μ.

If the probe is effective and hence oviposition may follow, the probability that a probe results in an oviposition is dependent only on j, the number of eggs already present in a host, say δ_j, $j=0, 1, 2, \ldots$

For the present it is assumed only that $1=\delta_0>\delta_1\geqq\delta_2\geqq\delta_3\geqq\ldots$ and that δ_0, δ_1, δ_2, ... do not change during the experiment.

The assumption that $\delta_0=1$ means that at the very first effective probe the parasite always lays an egg. The assumption that the series $\delta_0, \delta_1, \delta_2, \ldots$ is not ascending is the mathematical expression of the biological

Table 6. Details of examples presented in Fig. 1

Ex-ample	Model	No. of hosts	Mean $\bar{x}$	No. of eggs per host						$\hat{\lambda}$	$\hat{\delta}_1$	$\hat{\delta}_2$	χ^2	P
				0	1	2	3	4	5+[a]					
a	Obs.	60	0.72	24	30	5	1	0	0	—	—	—	—	—
	Poiss.			29.2	21.0	7.7	1.0	0.2	0.0	—	—	—	6.56	0.087
	I			23.9	29.6	5.7	0.7	0.1	0.0	0.918	0.382	—	0.29	0.86
b	Obs.	49	1.10	8	30	9	2	0	0	—	—	—	—	—
	Poiss.			15.9	18.0	9.9	3.6	1.0	0.2	—	—	—	12.92	0.011
	I			8.0	29.9	9.2	1.7	0.2	0.0	1.816	0.280	—	0.31	0.85
c	Obs.	86	1.58	3	36	41	6	0	0	—	—	—	—	—
	Poiss.			17.8	28.0	22.2	11.6	4.6	1.5	—	—	—	39.32	<0.001
	I			2.5	42.3	27.7	10.2	2.7	0.6	3.556	0.265	—	12.46	0.002
	II			3.0	35.9	41.2	5.5	0.5	0.0	3.360	0.354	0.090	0.57	0.45
d	Obs.	91	2.04	1	23	40	25	2	0	—	—	—	—	—
	Poiss.			11.7	24.0	24.6	16.8	8.6	4.8	—	—	—	33.33	<0.001
	I			0.7	29.6	31.7	18.5	7.5	3.1	4.928	0.291	—	13.25	0.004
	II			0.9	22.1	43.1	18.4	5.2	1.3	4.589	0.408	0.205	5.90	0.05
	III			1.0	21.9	43.1	20.3	4.2	0.5	4.521	0.418	—	2.99	0.41
	IV			1.3	21.0	43.9	21.5	3.1	0.2	4.266	0.465	—	1.76	0.62
e	Obs.	88	2.30	1	12	42	28	5	0	—	—	—	—	—
	Poiss.			8.8	20.3	23.3	17.9	10.3	7.3	—	—	—	41.04	<0.001
	I			0.5	24.1	29.6	19.9	9.3	4.6	5.088	0.326	—	22.56	<0.001
	II			0.9	11.5	45.2	21.9	6.7	1.8	4.550	0.616	0.216	4.26	0.12
	III			0.8	14.4	39.5	25.3	6.9	1.1	4.710	0.515	—	2.57	0.47
	IV			0.2	11.5	42.4	28.0	4.7	0.2	6.036	0.421	—	0.49	0.92
f	Obs.	109	2.42	1	10	51	40	6	1	—	—	—	—	—
	Poiss.			9.5	23.2	28.1	22.7	13.7	10.3	—	—	—	59.69	<0.001
	I			0.5	27.2	35.9	25.7	12.8	6.9	5.442	0.322	—	34.33	<0.001
	II			0.9	9.3	56.4	29.8	9.7	2.8	4.795	0.712	0.211	6.69	0.04
	III			0.7	14.4	47.0	34.4	10.6	2.0	5.080	0.527	—	6.28	0.09
	IV			0.1	10.3	49.7	39.8	7.8	0.5	6.875	0.419	—	1.23	0.75

[a] 5+ means "5 or more".

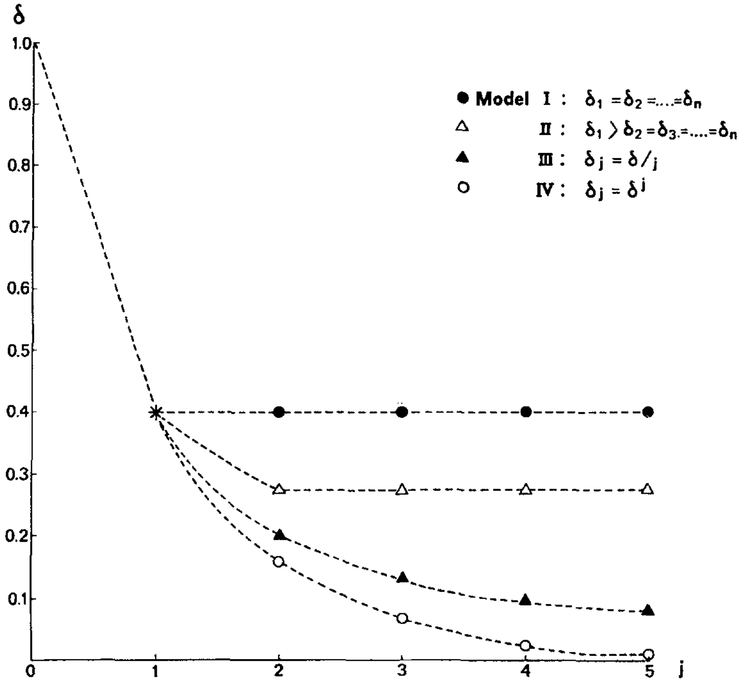

Fig. 2. Comparison of the changes in the chance δ that an egg will be laid in a host containing j eggs in the four models. (The dotted line is only drawn for convenience of reading the graph.) The values for δ_1 and δ_2 (model II) are arbitrarily chosen

notion that it would be very unlikely if the parasite would prefer hosts containing many eggs over hosts with fewer eggs.

Finally, we denote with $Y_i, i=1, \ldots, n$ the number of eggs present in larva (i) at the end of the experiment. In the Appendix, part B, one can find a derivation of the probability distribution of this random variable for the general case. In part C it is further elaborated for the four models.

The total number of observations consists of 119 sets of n observed values y_i, $i=1, \ldots, n$.

All estimates and tests are performed with these figures. For the details one is referred to the Appendix, part D and E.

Model I

The first model was already described in Bakker *et al.* (1967), and it supposed that $\delta_0=1$, and $\delta_1=\delta_2=\delta_3 \cdots = \delta < 1$; λ and δ are unknown. In model I it was assumed that the wasp is only able to distinguish whether the host larva had been parasitized or not, but that it is absolutely unable to distinguish whether 1, 2, or 3, . . . eggs are present (Fig. 2).

The parameters λ and δ have been estimated from the observed distributions of eggs in all 47 experiments of set I a. The mathematical details can be found in the Appendix. The calculations of the estimates $\hat{\lambda}$ and $\hat{\delta}$ for each distribution have been executed on the IBM 360–50 of the "Centraal Rekeninstituut" by means of a numerical optimization technique in which both parameters were chosen in such a way that the weighted differences between the calculated and observed frequences were as small as possible: the chi-square-minimum method.

Under the hypothesis that the model under consideration is right, the random variable χ^2 follows approximately a chi-square distribution with a number of degrees of freedom which is equal to the number of observed classes minus one and minus the number of estimated parameters (2 in this model). The well-known "goodness of fit" test can then be applied.

Table 7. Comparison of estimates obtained by the chi-square-minimum method and the maximum likelihood method (model III)

	No. of eggs per host						$\hat{\lambda}$	$\hat{\delta}$	χ^2	P
	0	1	2	3	4	5+				
1. $\bar{x} = 1.48$										
Obs.	3	23	21	3	0	0				
χ^2 min	2.89	24.10	18.66	3.94	0.38	0.02	2.850	0.346	0.975	0.62
Max. lik.	2.90	24.55	18.41	3.77	0.35	0.02	2.847	0.336	0.993	0.61
2. $\bar{x} = 1.52$										
Obs.	4	22	18	6	0	0				
χ^2 min	3.99	21.23	19.28	4.88	0.57	0.04	2.527	0.470	0.976	0.62
Max. lik.	4.00	21.77	19.03	4.64	0.52	0.03	2.525	0.454	1.007	0.60
3. $\bar{x} = 1.56$										
Obs.	4	22	22	4	0	0				
χ^2 min	3.87	23.11	19.78	4.70	0.51	0.03	2.597	0.429	0.956	0.60
Max. lik.	3.88	23.57	19.54	4.50	0.48	0.03	2.594	0.417	0.984	0.61
4. $\bar{x} = 1.59$										
Obs.	1	18	29	2	0	0				
χ^2 min	0.80	20.63	21.93	5.88	0.70	0.05	4.130	0.282	5.978	0.05
Max. lik.	0.81	22.23	21.23	5.14	0.56	0.04	4.121	0.257	6.139	0.05
5[a]. $\bar{x} = 1.84$										
Obs.	0	19	21	9	1	0				
χ^2 min	0.04	17.54	23.88	7.46	1.00	0.08	7.218	0.171	0.904	0.83
Max. lik.	0.01	17.66	23.94	7.35	0.97	0.07	8.393	0.142	0.914	0.82

[a] Expt. with larvae of *D. funebris* (24 hours) as hosts.

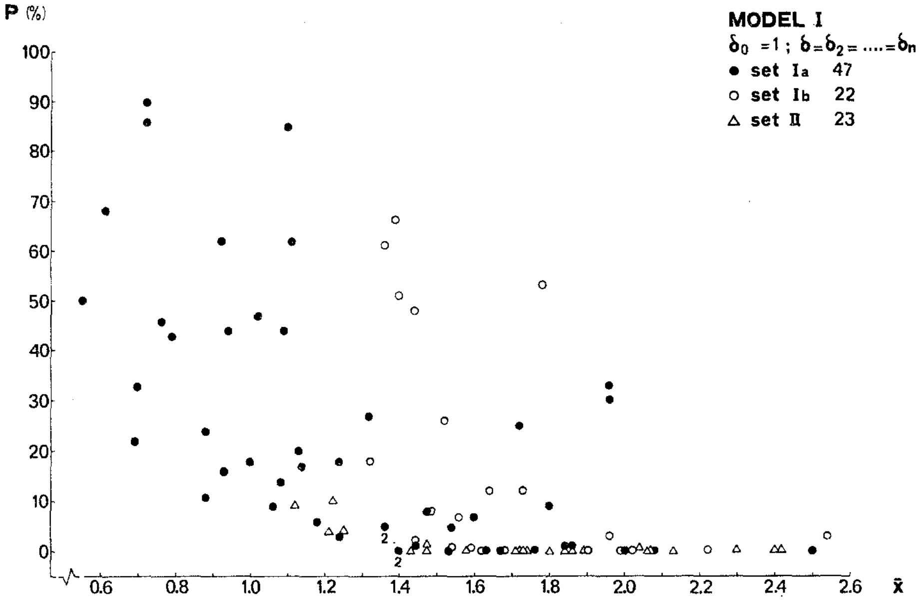

Fig. 3. P-values for the test statistic χ^2 on the assumptions of model I

This chi-square-minimum method for the estimation of λ and δ has been chosen in view of this goodness of fit test which has been applied. In order to get an impression whether the estimates would differ from those obtained with the maximum likelihood method to any appreciable degree, we have in a number of cases tried both methods. A few examples are given in Table 7 (for model III). It appears that the estimates do not differ very much.

The distributions a_1–f_1 in Fig. 1 illustrate the results obtained with this model with increasing $\bar{x}$ (see also Table 6). It is clear it gives a far better description of the distributions of the eggs than the Poisson distribution, at least at the lower values of $\bar{x}$. On the assumption that the model gives a good description of the set of observed distributions, the P-values will be uniformly distributed over the whole range between 0 and 100%.

Fig. 3 shows that for values of $\bar{x}$ (the mean number of eggs per host) up to about 1.1 the model gives a good description. (In this figure, also the P-values have been plotted for the 22 distributions of set Ib with a mean number of eggs per host $\bar{x}$ larger than 1.3, and for 23 out of 26 distributions of set II.)

These findings confirmed our earlier observations, and therefore a second model was developed.

Model II

In the second model it is assumed that

$$\delta_0=1,\ \delta_0>\delta_1>\delta_2,\ \delta_2=\delta_3=\delta_4,\ \ldots;\ \lambda,\ \delta_1,\ \delta_2$$

are unknown (model I is, hence, a special case of model II, in which $\delta_1=\delta_2$).

The second model expresses a situation in which the wasp is able to distinguish between the presence of only one egg in a host and the presence of two or more eggs (Fig. 2). The model was applied to 26 of the distributions of set Ia, and later to the 21 distributions of set Ib, in which $\bar{x}$ was larger than 1.30, as well as to 24 distributions of set II.

For illustration, three distributions obtained with model II are compared to the observed distributions in Fig. 1, c_2, d_2, f_2 (see also Table 6).

The chi-square test for goodness of fit has been applied on all these distributions, and the P-values have been plotted against $\bar{x}$ in Fig. 4. The test can, of course, not be applied in those cases where the number of observed classes minus one is the same as the number of parameters (3 in this model: λ, δ_1, δ_2). Therefore the number of cases in which the test statistic χ^2 could be calculated was lower than for model I. From Fig. 4 it will be immediately clear that model II gives a better description of the observed distributions than model I.

Under the assumption that model II (of which model I is a special case) is right, it is possible to test the hypothesis that $\delta_1=\delta_2$ with the aid of the so-called likelihood ratio test. Since the estimates obtained with the chi-square-minimum method and the maximum likelihood method do not differ very much, the former have been used as approximations for the latter to apply this test. This has been done for 50 distributions, and the results are given in Fig. 5. The hypothesis can be rejected in 37 cases ($P<5\%$).

However, turning again to Fig. 4, we see that at higher mean numbers of eggs per host ($\bar{x}>2.0$) 7 P-values out of 15 are $<5\%$, and $13<20\%$. Another disadvantage of the model is that the number of parameters to be estimated is rather high in relation to the number of classes observed, and that there is no strict relation between δ_1 and δ_2.

Because of these objections, we constructed a third model.

Model III

In the third model it is assumed that

$$\delta_0=1,\ \delta<1,\ \delta_1=\delta/1,\ \delta_2=\delta/2,\ \delta_3=\delta/3,\ \ldots,\ \delta_j=\delta/j,\ \delta_{j+1}=\delta/(j+1),\ \ldots;$$

λ and δ are unknown.

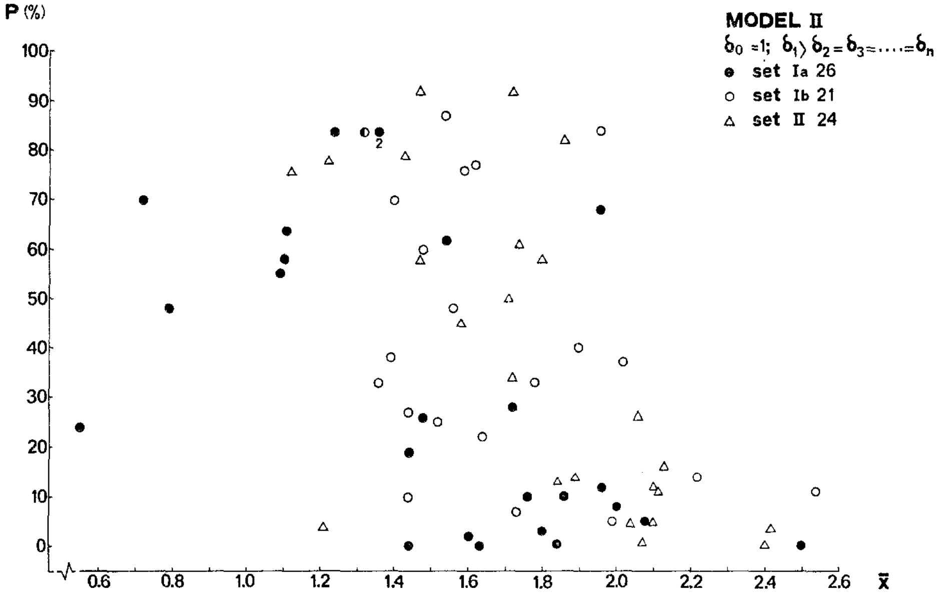

Fig. 4. P-values for the test statistic χ^2 on the assumptions of model II

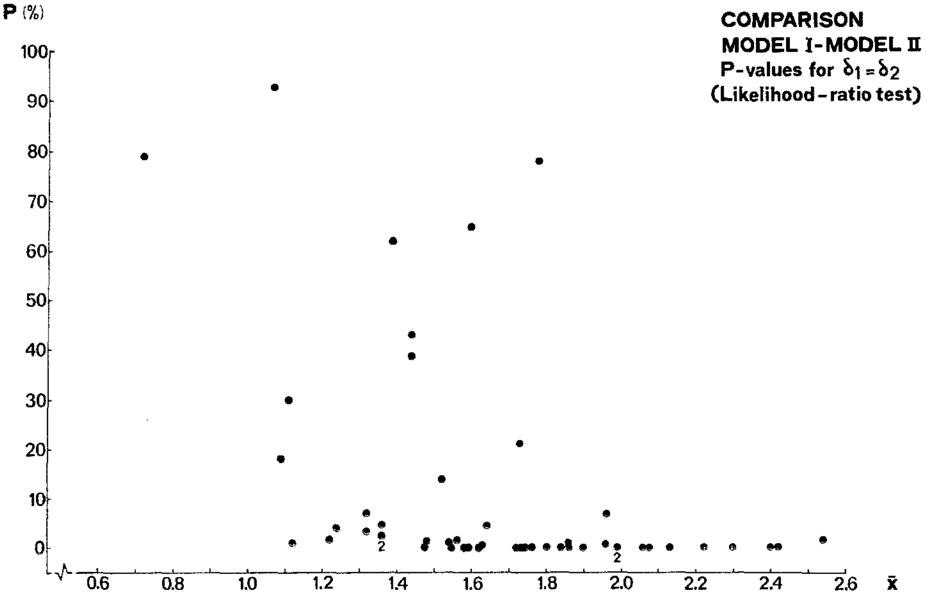

Fig. 5. Comparison of the models I and II. P-values for the test statistic χ^2 for testing the null-hypothesis $\delta_1 = \delta_2$ on the assumptions of model I

These assumptions express the situation that the wasp always prefers hosts with a lower number of eggs. The ratio of the chance that a female will lay an egg in a host larva in which already j_1 eggs have been laid to the chance that she will lay an egg in a larva containing j_2 eggs is inversely proportional to the number of eggs already present, *i. e.* $\delta_{j_1}:\delta_{j_2}=j_2:j_1$.

In this model $\delta_j:\delta_{j+1}=(j+1):j$, and, since

$$\lim_{j\to\infty}\frac{\delta_j}{\delta_{j+1}}=\lim_{j\to\infty}\frac{j+1}{j}=1,$$

this means that the wasps' ability to distinguish between host larvae containing increasing numbers of eggs diminishes rapidly (Fig. 2). This could be interpreted as follows: let us assume that at every oviposition the concentration of some substance which influences the chance that the parasite will lay an egg is increased by one unit. This hypothetical substance, be it injected into the host at oviposition, given off by the egg, or produced by the host after oviposition, will be present in the host at concentrations 0, 1, 2, 3, ... Now if the parasite distinguishes concentrations by their relative differences, model III will give a good description of the results that may be expected of her ovipositons. It should be emphasized that both in this model and model IV, the ability for discrimination δ is somehow fixed in each wasp. The discrimination shown at each actual visit is prescribed by the model: here $\delta_j=\delta/j$, $j=1, 2, 3, \ldots$.

This model was first applied to 42 distributions of set I b, and later to the 21 distributions of set I a with $\bar{x}>1.35$, to all 25 distributions of set II, and to the 4 distributions of set III. In Fig. 1 three distributions (d_3, e_3, f_3) have been used to compare model III with the observed distributions for illustration (see also Table 6). Again, the chi-square test for goodness of fit has been applied on all distributions and Fig. 6 gives the P-values plotted against $\bar{x}$.

Up to values for $\bar{x}$ of about 1.4, model III describes the observed distributions satisfactorily. At higher values it is certainly better than model I, and, at least for values above 2.0, also better than model II. However, for $\bar{x}>1.4$ out of a total of 60 P-values, 27 are $<5\%$. We had the impression that the chances for another egg decreased more rapidly than in model III: *i. e.*, the expected frequencies of hosts with 4 or more parasite eggs was still too high in model III. Therefore, a fourth model was developed.

Model IV

In the fourth model it is assumed that

$$\delta_0=1,\ \delta<1,\ \delta_1=\delta,\ \delta_2=\delta^2,\ \delta_3=\delta^3,\ \ldots,\ \delta_j=\delta^j,\ \delta_{j+1}=\delta^{j+1},\ \ldots;$$

λ and δ are unknown.

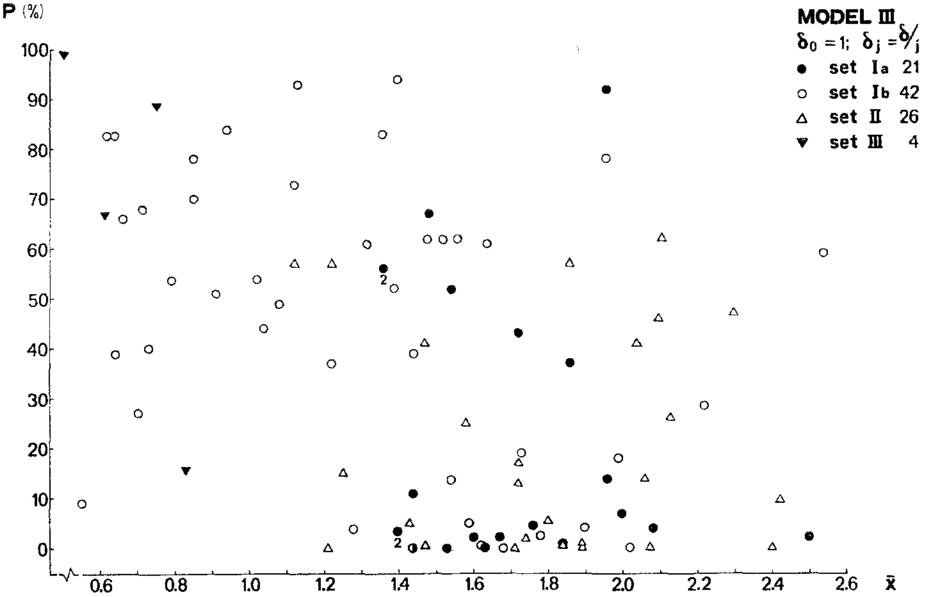

Fig. 6. P-values for the test statistic χ^2 on the assumptions of model III

In this model the ratio $\delta_j : \delta_{j+1} = 1 : \delta$, which means that for $j = 0, 1, 2, 3, \ldots$ the female wasp has the same larger readiness to lay an egg in a host with j eggs compared to a host with $j+1$ eggs. The ability to distinguish between host larvae containing different numbers of eggs remains the same, independent of the number present. This means that the wasp must be able to distinguish the supposed concentrations by their absolute differences. A biological translation of this model would be that during the experiment the threshold for oviposition is lowered in such a way, that the same discrimination which the female first showed between hosts with one and with two eggs, shifts when $\bar{x}$ increases to hosts with two and with three eggs, etc.

In comparison to model III, δ in this model decreases far more rapidly as a function of j (Fig. 2). For all 26 distributions of set II, and later also 21 of set Ia ($\bar{x} > 1.35$) as well as 22 of set Ib ($\bar{x} > 1.30$) and 4 of set III, λ and δ were estimated according to this model, the expected frequencies were calculated and again the goodness of fit test was applied. Three distributions obtained with model IV are compared to the observed distributions in Fig. 1, d_4, e_4, f_4, for illustration (see also Table 6). In Fig. 7, for all distributions the P-values are plotted against $\bar{x}$. Of 60 P-values for distributions with $\bar{x} > 1.4$, 14 are $< 5\%$. In Table 8 the models III and IV are compared as regards "goodness of fit". For all

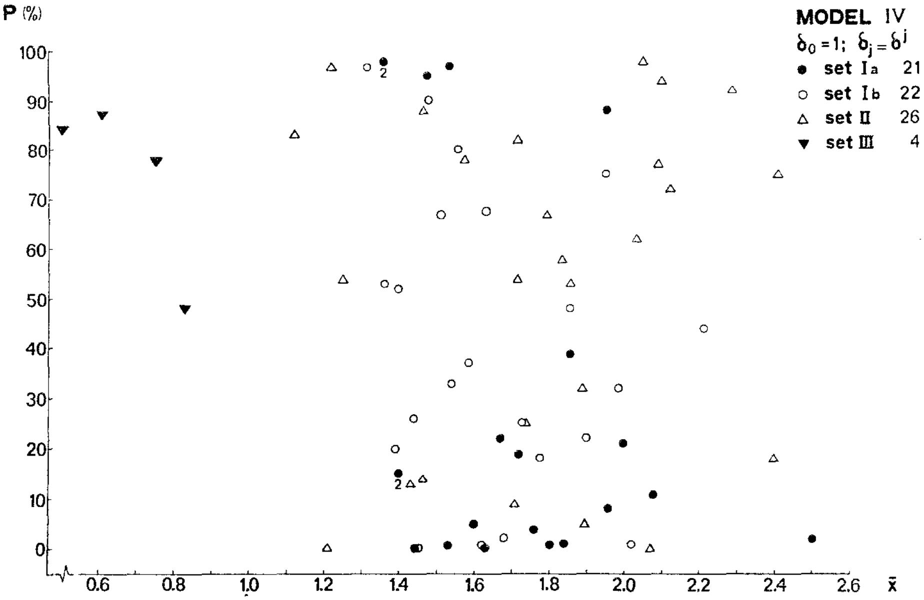

Fig. 7. P-values for the test statistic χ^2 on the assumptions of model IV

Table 8. Comparison of the "goodness of fit" of model III and model IV for 60 distributions

Model III	Model IV			
	$P<5\%$	$5\%<P<10\%$	$P>10\%$	Total
$P<5\%$	11	3	11	25
$5\%<P<10\%$	—	—	5	5
$P>10\%$	—	1	29[a]	30
Total	11	4	45	60

[a] 22 P-values increase. 7 P-values decrease.

60 distributions in which $\bar{x}>1.4$ the changes in P-values have been summarized. Of all 25 cases in which P is smaller than 5% for model III, P becomes larger than 10% in 11 cases, and between 5 and 10% in 3 cases. In those 5 cases in which P for model III is between 5 and 10%, P becomes larger than 10% in model IV. In 30 cases P is larger than 10% for model III. Of these, 22 increase for model IV; 8 decreased, of which 1 becomes below 10%. It is obvious that, of all models tested, model IV gives the best description of the distributions of eggs found. Fig. 8

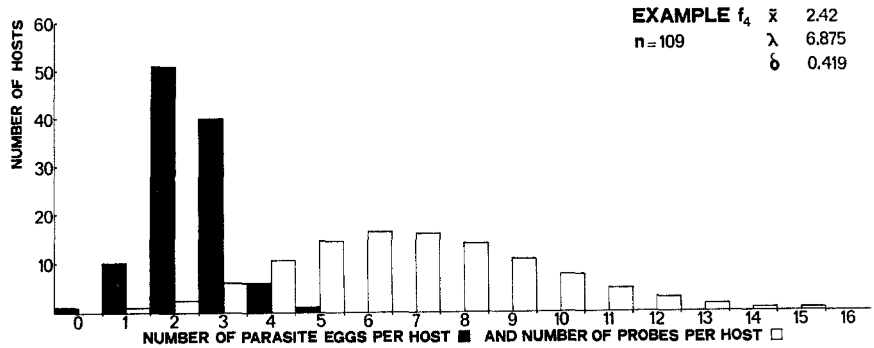

Fig. 8. Frequency distribution of the number of eggs per host ($\bar{x}$, example f of Fig. 1) and the estimated number of effective probes per host (Poisson distribution with mean $\hat{\lambda}$) according to model IV

illustrates the frequency distribution of the "effective probes" according to model IV, resulting in the frequency distribution of the eggs laid in example f_4 (Fig. 1). It is clear that in order to produce the observed distribution of 264 eggs over 109 hosts, the mean number of effective probes must have been very high ($\hat{\lambda}=6.875$).

V. Discussion

The models presume that δ is a property of the egg-laying female. It should therefore be independent of λ, the expected mean number of effective probes. This was checked by calculating the regression of $\hat{\delta}$ on $\hat{\lambda}$ for the best-fitting models III and IV (Table 9, Figs. 9 and 10). The

Table 9. Linear regression between $\hat{\lambda}$ and $\hat{\delta}$, $\hat{\lambda}$ and $\bar{x}$, and $\bar{x}$ and $\hat{\delta}$, for models III and IV

	Intercept	Regression coefficient	t-value	P
Model III: 90 observations				
$\hat{\lambda}$, $\hat{\delta}$	0.28881	0.019062	1.516750	0.07
$\hat{\lambda}$, $\bar{x}$	0.46615	0.331089	17.716705	<0.001
$\bar{x}$, $\hat{\delta}$	0.09988	0.164214	5.617139	<0.001
Model IV: 73 observations				
$\hat{\lambda}$, $\hat{\delta}$	0.42018	—0.012874	—0.984933	0.12
$\hat{\lambda}$, $\bar{x}$	0.82189	0.227077	10.854774	<0.001
$\bar{x}$, $\hat{\delta}$	0.08658	0.172391	4.209242	<0.001

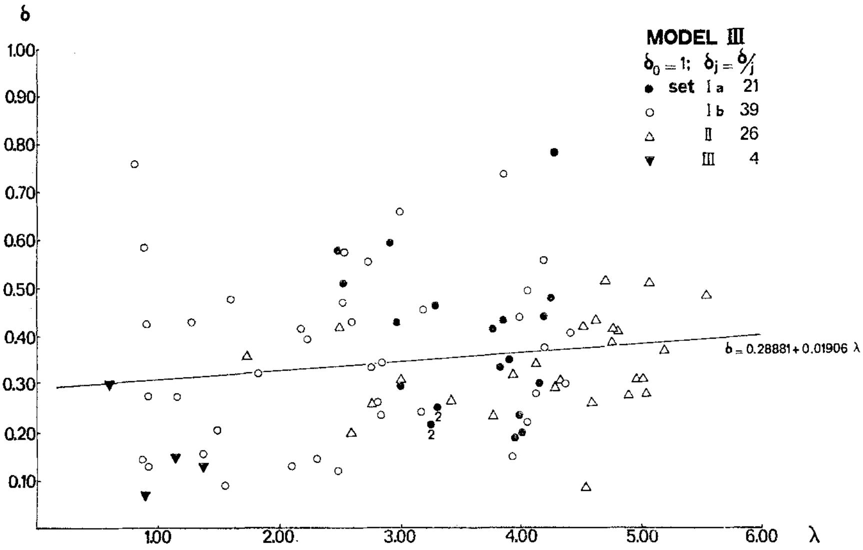

Fig. 9. Model III, regression of $\hat{\delta}$ on $\hat{\lambda}$

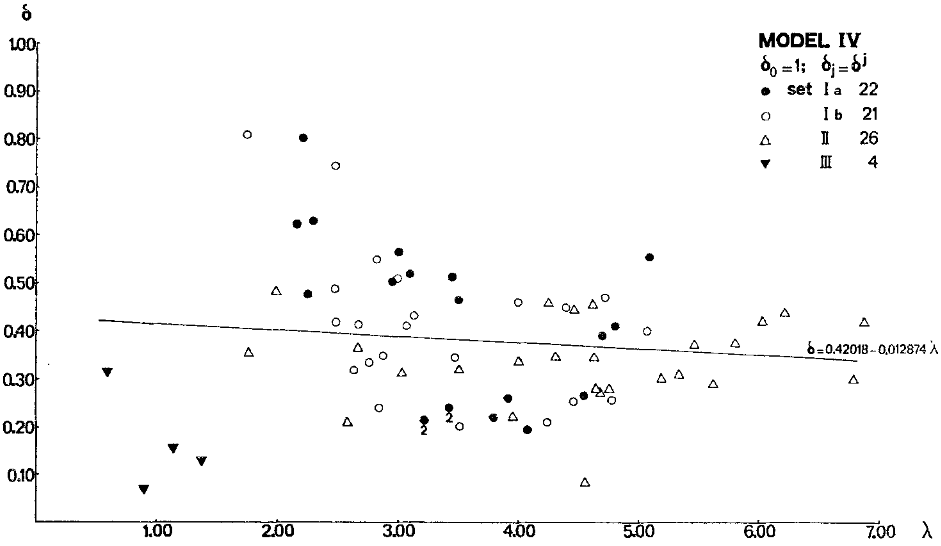

Fig. 10. Model IV, regression of $\hat{\delta}$ on $\hat{\lambda}$

regression coefficient is not significant, very small, and even of different sign in the two cases. In our first paper on the subject of discrimination by *Pseudeucoila* (Bakker *et al.*, 1967) we found a clear positive correlation between $\hat{\lambda}$ and $\hat{\delta}$. This was due to the fact that we then erroneously assumed that the wasp was only able to distinguish parasitized from unparasitized hosts, but that it was absolutely unable to distinguish whether 1, or 2, or 3, . . . eggs were present (model I).

It should be mentioned that the δ's estimated in our experiment do not refer to individual females, but to a varying number of females (generally 4). Nevertheless, the resultant $\hat{\delta}$'s appear to vary very much.

Obviously there is a strong positive correlation between $\hat{\lambda}$ and $\bar{x}$, the mean number of eggs laid (Table 9). The great variability in δ will result in different mean numbers of eggs laid ($\bar{x}$) at the same or similar expected mean number of effective probes (λ). This causes a positive correlation between $\bar{x}$ and $\hat{\delta}$ (Table 9, Figs. 11 and 12).

The fact that model IV gives a fairly good description does not mean that no other models might be developed which describe the distribution of the parasite eggs over the host larvae equally well or even better. It should be realized that the value of descriptive models like the ones discussed is limited as long as the process producing the distributions is not very well known. Indeed, a description of the complex process of oviposition and discrimination with the aid of only two parameters can only be rather primitive. Of course, the models were developed on the basis of quite a large number of characteristics of the parasites, but most characteristics of its behaviour are only known qualitatively and some very important data are even still lacking. For instance, it is essential to have direct estimates of p and μ (and hence of λ) and of δ in order to compare them with the values estimated from the observed distribution of eggs, according to the models discussed in this paper. However, these data appear to be very difficult to obtain, at least for high λ's because it is without special techniques (videotape) impossible to observe the behaviour of more than one female wasp at a time, and because one wasp will not oviposit more than once in each of a limited number of hosts (*e. g.* 25) within an observation period of 3 hours, although the hosts are hit on the average quite a few times, judging from the total number of hits observed. It is practically impossible to increase this period for one observer, but we intend to work in "shifts" to obtain the necessary information. Further it is necessary to record the hits of the wasp of every individual host larva separately, and this is only possible when the host larvae are restricted in their movements. This can, for instance, be accomplished by placing individual hosts in small circular spots of yeast suspension, arranged in a hexagonal pattern on an agar base. The hosts

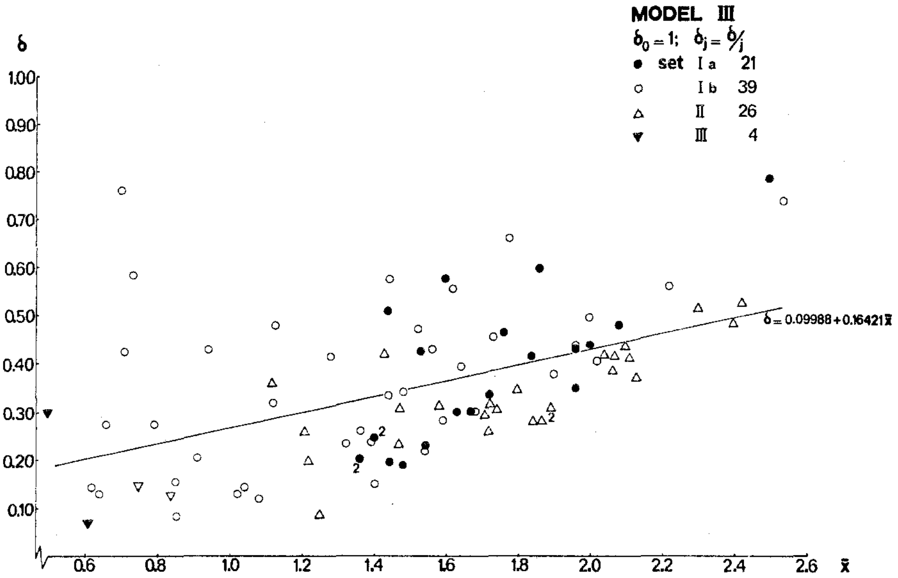

Fig. 11. Model III, regression of $\hat{\delta}$ on $\bar{x}$

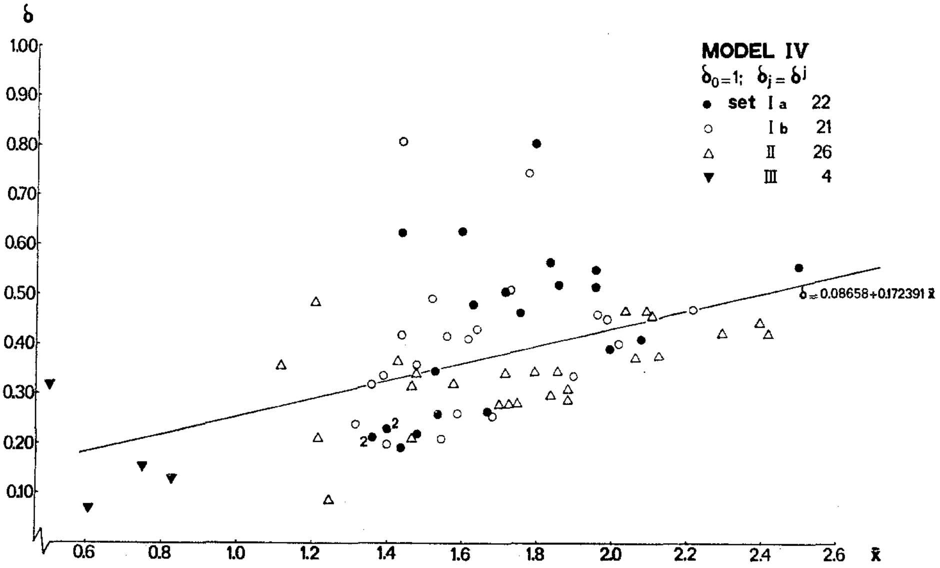

Fig. 12. Model IV, regression of $\hat{\delta}$ on $\bar{x}$

will remain in their spots and the visits of the parasites to each of them can be recorded. Experiments with this set-up are under way.

We expect they will give us quantitative information on a complex of factors that determine parasitization, *e. g.* walking speed, locomotion pattern, time spent on each yeast spot, frequency of pricks of the ovipositor into the host medium, ratio of pricks to hits, of hits to probes, and of probes to ovipositions, all in relation to unparasitized and parasitized hosts at different densities.

Further we hope to obtain data on the time needed for building up the factor which causes avoidance of superparasitization.

Finally we intend to try and develop a simulation model, and feed it with all relevant parameters until it will predict the distribution of parasite eggs over the host larvae to a satisfactorily accurate degree.

The biological significance of the ability to discriminate between parasitized and unparasitized hosts is obvious. However, it is less easy to see the significance of the ability to distinguish hosts with $(j+1)$ eggs from hosts with j eggs and to possess a lower tendency to lay eggs in the former in all cases where $j>0$ in a wasp like *Pseudeucoila*, in which one host never produces more than one parasite. Obviously, the ability may have no function, but we have some indications that there is an increasing tendency of females to migrate from an environment in which only parasitized hosts are found. We will report on these experiments later, but if this tendency to migrate from a place where only parasitized larvae are found is increased by the degree of superparasitization, then the ability to distinguish different degrees of parasitization would have some significance.

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Mrs. A. van der Waals-Straatman and Miss M. van Wijngaarden are acknowledged for the conscientious typework.

We had a number of fertile discussions with Mr. J. A. J. Metz. Dr. Nora Croin Michielsen read the manuscript and suggested a number of improvements.

Appendix

A. Let Z' be the number of times that a larva is probed. It is assumed that Z' is a random variable having a Poisson distribution with expectation μ. Each probe is effective with probability p independent of all earlier probes. Hence, Z, the number of effective probes on the condition that the larva is probed z times is binomially distributed with parameters z and p. We shall prove now the well-known fact that Z, the number of effective probes (unconditional), is a random variable having a Poisson distribution with expectation $\lambda = p.\,\mu$. The probability that Z is equal to z

is $P\ (Z=z)=P\ (Z'=z$ and all probes are effective$)+P\ (Z'=z+1$ and all probes but one are effective$)+P\ (Z'=z+2$ and all probes but two are effective$)+\ldots=$

$$e^{-\mu}\frac{\mu^z}{z!}\cdot\binom{z}{z}p^z(1-p)^0+e^{-\mu}\frac{\mu^{z+1}}{(z+1)!}\binom{z+1}{z}p^z(1-p)^1+e^{-\mu}\frac{\mu^{z+2}}{(z+2)!}\binom{z+2}{z}\cdot$$

$$p^z(1-p)^2+\cdots=e^{-\mu}\frac{(p\mu)^z}{z!}\sum_{k=0}^{\infty}\frac{\{\mu(1-p)\}^k}{k!}=e^{-\mu}\frac{(p\mu)^z}{z!}e^{\mu(1-p)}=e^{-\lambda}\frac{\lambda^z}{z!},$$

and this is true for $z=0, 1, 2, \ldots$

B. Let us consider a randomly chosen larva. It is assumed that it is probed effectively Z times and that Z follows a Poisson distribution with expectation λ. The probability that an effective probe is successful in the sense that the probe results in oviposition is δ_j for $j=0, 1, 2, \ldots$, where j is the number of eggs already present in the larva. Furthermore it is assumed that $\delta_0=1$. Let Y denote the number of eggs present in the larva at the end of the experiment. This random variable has a probability distribution denoted by $P(Y=y)$ for $y=0, 1, 2, \ldots$. The probability $P(Y=0)$ that the larva remains empty, is equal to the probability that the larva is not probed effectively, hence

$$P(Y=0)=P(Z=0)=e^{-\lambda}. \tag{1}$$

The probability $P(Y=1)$ that the larva contains exactly one egg at the end of the experiment is equal to the probability that it is probed one time effectively and of course successfully because $\delta_0=1$ or two times of which only the first one was successful, or three times of which only the first one was successful, or $\ldots$, etc. Since the events "z effective probes and only the first one was successful" are disjoint events for $z=1, 2, 3, \ldots$, it is true that

$$P(Y=1)=e^{-\lambda}\lambda+e^{-\lambda}\frac{\lambda^2}{2!}(1-\delta_1)+e^{-\lambda}\frac{\lambda^3}{3!}(1-\delta_1)^2+\cdots$$

$$=e^{-\lambda}\left[\frac{1}{1-\delta_1}\left\{1+\frac{\lambda(1-\delta_1)}{1!}+\frac{\{\lambda(1-\delta_1)\}^2}{2!}+\frac{\{\lambda(1-\delta_1)\}^3}{3!}+\cdots\right\}-\frac{1}{1-\delta_1}\right]$$

$$=\frac{e^{-\lambda\delta_1}}{1-\delta_1}+\frac{e^{-\lambda}}{\delta_1-1} \tag{2}$$

The probability $P(Y=2)$ that the larva contains exactly two eggs at the end of the experiment is equal to the probability that it is probed effectively two times, both probes successful or three times, of which only the first and the second probe were successful or three times, of which only the first and the third probe were successful, or four times, of which only the first and the second were successful, etc., etc.

Since these events are again disjoint events, it is true that

$$P(Y=2)=e^{-\lambda}\frac{\lambda^2}{2!}\delta_1+e^{-\lambda}\frac{\lambda^3}{3!}\{\delta_1(1-\delta_2)+(1-\delta_1)\delta_1\}$$

$$+e^{-\lambda}\frac{\lambda^4}{4!}\{\delta_1(1-\delta_2)^2+(1-\delta_1)\,\delta_1(1-\delta_2)+(1-\delta_1)^2\delta_1\}+\cdots$$

which may be shown to be equal to

$$\delta_1\left\{\frac{e^{-\lambda\delta_2}}{(1-\delta_2)\,(\delta_1-\delta_2)}+\frac{e^{-\lambda\delta_1}}{(1-\delta_1)\,(\delta_2-\delta_1)}+\frac{e^{-\lambda}}{(\delta_1-1)\,(\delta_2-1)}\right\}. \tag{3}$$

In exactly the same way one can derive the probabilities that $Y=3, 4, 5, \ldots$. The probability that $Y=y$ equals

$$P(Y=y) = \sum_{k=0}^{\infty} e^{-\lambda} \frac{\lambda^{y+k}}{(y+k)!} \sum_{n_1} \sum_{n_2} \cdots \sum_{n_y} (1-\delta_1)^{n_1} \delta_1 (1-\delta_2)^{n_2} \delta_2 \cdots$$
$$(1-\delta_{y-1})^{n_{y-1}} \delta_{y-1} (1-\delta_y)^{n_y}$$

where the summations range over all possible non-negative integer values of n_1, $n_2, \ldots, n_y$ on the condition that $n_1+n_2+\cdots+n_y=k$. It can be shown that $P(Y=y)$ for $y=1, 2, 3, \ldots$ may be written as a finite sum:

$$\begin{aligned} P(Y=y) = \delta_1 \delta_2 \cdots \delta_{y-1} \Bigg\{ & \frac{e^{-\lambda \delta_y}}{(1-\delta_y)(\delta_1-\delta_y)\cdots(\delta_{y-1}-\delta_y)} \\ & + \frac{e^{-\lambda \delta_{y-1}}}{(1-\delta_{y-1})(\delta_1-\delta_{y-1})\cdots(\delta_{y-2}-\delta_{y-1})(\delta_y-\delta_{y-1})} + \cdots \\ & + \frac{e^{-\lambda \delta_1}}{(1-\delta_1)(\delta_2-\delta_1)\cdots(\delta_y-\delta_1)} + \frac{e^{-\lambda}}{(\delta_1-1)(\delta_2-1)\cdots(\delta_y-1)} \Bigg\}. \end{aligned} \tag{4}$$

The probability distribution of Y is now completely determined by (1) and (4).

An alternative way of reasoning, of course leading to the same result, is the following. Let us consider the probes in a larva during the experiment as the events in a Poisson process. This means that the total number of effective probes during the experiment is Poisson distributed. The probability that a probe will be successful is δ_j for $j=0, 1, 2, \ldots$.

If we consider merely the process of successful effective probes, then it is to describe as a "pure-birth" process with parameters $\lambda\delta_0$, $\lambda\delta_1$, $\lambda\delta_2, \ldots$. Following Feller (1962, 1966) one can derive the same expression for the probability distribution of Y.

In section IV it is assumed that $Z'_1, \ldots, Z'_n$ are mutually independent; the corresponding $Y_1, \ldots, Y_n$ are hence mutually independent too and the observation $y_1, \ldots, y_n$ of observed numbers of eggs in the n different larvae can be considered as a sample of size n of a random variable having a probability distribution $P(Y=y)$ for $y=0, 1, 2, \ldots$.

C. In model I it is assumed that $\delta_1=\delta_2=\cdots=\delta$. By taking the limit, or by taking an alternative derivation (4) may be shown to be equal to

$$\begin{aligned} P(Y=y) = \delta^{y-1} \Bigg[e^{-\lambda\delta} \Bigg\{ & \frac{\lambda^{y-1}}{(1-\delta)(y-1)!} - \frac{\lambda^{y-2}}{(1-\delta)^2(y-2)!} \\ & + \cdots (-1)^{y-2} \frac{\lambda}{(1-\delta)^{y-1}} + (-1)^{y-1} \cdot \frac{1}{(1-\delta)^y} \Bigg\} + (-1)^y \frac{e^{-\lambda}}{(1-\delta)^y} \Bigg]. \end{aligned} \tag{5}$$

Formula (5) is mentioned in Bakker *et al.* (1967) in a slightly different notation.

In model II it is assumed that $\delta_2=\delta_3=\delta_4=\ldots$. The probabilities that the number of eggs present in a larva is zero or one or two are given respectively by the formulae (1), (2), and (3). The explicit expression for the probabilities that Y is three or four are:

$$\begin{aligned} P(Y=3) = \delta_1 \delta_2 \Bigg[& e^{-\lambda\delta_2} \Bigg\{ \frac{\lambda}{(1-\delta_2)(\delta_1-\delta_2)} + \frac{2\delta_2-\delta_1-1}{(1-\delta_2)^2(\delta_1-\delta_2)^2} \Bigg\} \\ & + \frac{e^{-\lambda\delta_1}}{(1-\delta_1)(\delta_2-\delta_1)^2} + \frac{e^{-\lambda}}{(\delta_1-1)(\delta_2-1)^2} \Bigg]. \end{aligned} \tag{6}$$

$$P(Y=4)=\delta_1\,\delta_2^2\left[e^{-\lambda\delta_2}\left\{\frac{\lambda^2/2}{(1-\delta_2)\,(\delta_1-\delta_2)}+\frac{\lambda(2\,\delta_2-\delta_1-1)}{(1-\delta_2)^2\,(\delta_1-\delta_2)^2}\right.\right. \tag{7}$$

$$\left.\left.+\frac{\delta_1^2+3\,\delta_2^2-3\,\delta_1\,\delta_2+\delta_1-3\,\delta_2+1}{(1-\delta_2)^3\,(\delta_1-\delta_2)^3}\right\}+\frac{e^{-\lambda\delta_1}}{(1-\delta_1)\,(\delta_2-\delta_1)^3}+\frac{e^{-\lambda}}{(\delta_1-1)\,(\delta_2-1)^3}\right].$$

A more or less simple explicit expression for the general case does not seem to be available. Since the observed frequency of five or more eggs was always zero or very small, no attention is paid to that problem.

In model III and model IV it is assumed that $\delta_j=\delta/j$ and $\delta_j=\delta^j$, $j=1, 2, 3, \ldots$, respectively. Substitution in (4) does not give expressions which may be simplified essentially.

D. The unknown parameters are estimated by means of the so-called chi-square-minimum method. Let n be the total number of larvae in the experiment and define f_j for $j=0, 1, \ldots, J—1$, as the fraction of the n larvae containing j eggs at the end of the experiment. Define f_j as the fraction of the n larvae containing at least J eggs. Finally, define $p_j=P(Y=j)$ for $j=0, 1, \ldots, J—1$ and

$$p_J=\sum_{k=J}^{\infty}P(Y=k) \quad \text{and} \quad \chi^2=\sum_{j=0}^{J}\frac{(nf_j-np_j)^2}{np_j}.$$

The p_j are functions of the unknown parameters λ and δ or λ, δ_1 and δ_2, so χ^2 is a function of these parameters too. The two or three estimates are chosen in such a way that χ^2 is as small as possible for those values. This is accomplished by means of a numerical optimization procedure carried out on the 360–50 IBM computer from the "Centraal Rekeninstituut" of the University of Leiden. For testing the hypothesis $\delta_1=\delta_2$ in model II, one needs maximum likelihood estimates, both under the assumption of the null hypothesis as well as under the alternative hypothesis. That means, that the estimates are chosen—again by means of a numerical optimization procedure—in such a way that

$$\prod_{j=0}^{\infty}(\tilde{p}_j)^{nf_j}$$

attains a maximum for all possible and admissible values of the parameters, where $\tilde{p}_j$ is defined by $\tilde{p}_j=P(Y=j)$ for $j=0, 1, 2, \ldots$.

E. For a detailed description of the chi-square test for goodness of fit one is referred to one of the standard textbooks on mathematical statistics; see for instance Rao (1965). Under the assumption that the model is correct, the test statistic χ^2 follows approximately a chi-square distribution with $(J+1)—1—$ (the number of estimated parameters) $=J—2$ or $J—3$ degrees of freedom.

Only in one case it was possible to formulate one model as a special case of an other model: model I is a special case of model II for $\delta_1=\delta_2$. In such a case it is possible to test the hypothesis that $\delta_1=\delta_2$ under the assumption that model II is a correct model for describing the frequency distributions, by means of the likelihood ratio test. Let us denote the maximum likelihood estimates of λ, δ_1 and δ_2 under the assumptions of model II by λ^*, δ_1^* and δ_2^*, and let us denote the corresponding estimates under the hypothesis that $\delta_1=\delta_2=\delta$ by $\hat{\lambda}$ and $\hat{\delta}$. The two estimates λ^* and $\hat{\lambda}$ need not to be exactly equal of course. The probabilities $\tilde{p}_j$, $j=0, 1, 2, \ldots$, are functions of the parameters λ, δ_1 and δ_2 or λ and δ.

In order to indicate that the maximum likelihood estimates are substituted for these parameters values, we introduce the notation $\tilde{p}_j(\lambda^*, \delta_1^*, \delta_2^*)$ and $\tilde{p}_j(\hat{\lambda}, \hat{\delta})$ for

$j=0, 1, 2, \ldots$. The test statistic of the likelihood ratio test is then defined by

$$\Lambda = -2 \left[\log \prod_{j=0}^{\infty} \{\tilde{p}_j (\hat{\lambda}, \hat{\delta})\}^{nf_j} - \log \prod_{j=0}^{\infty} \{\tilde{p}_j (\lambda^*, \delta_1^*, \delta_2^*)\}^{nf_j} \right]. \qquad (8)$$

Under the assumptions of model II, and the hypothesis that $\delta_1 = \delta_2$, the statistic Λ follows approximately a chi-square distribution with one degree of freedom. For further details one is referred to textbooks on mathematical statistics, such as Wilks (1962) and Rao (1965). Actually we did not substitute the maximum likelihood estimates in (8) but the chi-square-minimum estimates. As is illustrated in Table 7, the differences are very small.

In testing several hypotheses in a large set of observations, great care is needed. Therefore the observed frequency distributions are divided into subsets and each hypothesis is tested on a new set of observations. The observations already used in one of the tests, are used for trying out new ideas and for checking the expected improvement of the fit. All results obtained in this way are thus independently tested using new observations.

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