Analysing Experiments on Frequency-Dependent Selection by Predators

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ANALYSING EXPERIMENTS ON FREQUENCY-DEPENDENT SELECTION BY PREDATORS

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SUMMARY

(1) Predators sometimes eat disproportionately more of the more abundant forms of their prey, thus promoting diversity in the prey population.

(2) Such selection may be investigated by exposing a series of prey populations with different relative frequencies of the various types to a series of predators.

(3) We use a simple model to describe the outcome of such a series of trials, relating the relative frequencies of the various prey types eaten to the relative frequencies available. Frequency-independent and frequency-dependent components of selection are separated in the model.

(4) Previous criticism of the model does not appear to be valid. An alternative model due to Manly seems *a priori* unrealistic and potentially misleading.

(5) We have investigated the fit of our model to all the available data. In most cases it is good. Manly's model is also a good fit to most of the data, except at extreme prey frequencies.

(6) Experiments of the type considered need to be carefully designed if the results from different experiments are to be comparable.

(7) They are more useful than most of the other types of experiment and observation that have been used for investigating frequency-dependent selection.

(8) Appendix 1 describes two techniques for fitting our model to the data from experiments with two prey types.

(9) Appendix 2 describes an approximate technique for fitting our model to the data from experiments with more than two prey types and illustrates it with a worked example.

INTRODUCTION

The idea that animals may tend to eat disproportionately more of the more abundant forms of their prey has long interested both ecologists and geneticists (Poulton 1884; Elton 1927; Cain & Sheppard 1954; Haldane 1955) to whom it is known as 'switching' (Murdoch 1969) and 'apostatic selection' (Clarke 1962) respectively. Since it promotes diversity in the prey populations, it might explain some of the bewildering diversity that is observed at both the ecological and genetical levels.

Such positive frequency-dependent selection by predators, first clearly demonstrated by J. A. Allen (Allen & Clarke 1968; Allen 1976), has been variously investigated. Perhaps the most illuminating work comprises those experiments in which prey of two or more types is exposed at several relative frequencies to a series of predators (Popham

1941, 1943; Landenberger 1968; Murdoch 1969; Manly, Miller & Cook 1972; Murdoch & Marks 1973; Lawton, Beddington & Bonser 1974; Cook & Miller 1977). The data are then examined for signs that the strength of the selection observed varies with frequency, being greatest at extreme frequencies and reversed at opposite extreme frequencies, so that the rare form tends to be protected by its rarity. If one wishes merely to test for the presence of frequency-dependent selection a simple correlation approach can be used. If e_1 and e_2 are the numbers of two prey types eaten from a population of A_1 and A_2 available, then the ratio e_1A_2/e_2A_1 will remain constant when A_1/A_2 is varied unless selection is frequency-dependent. If rank correlation is used, then no assumption is needed about the parametric form of any frequency-dependence. This was essentially the method used by Manly, Miller & Cook (1972).

We may wish to take the analysis further and estimate parameters of the selective process. To do this, we require adequate models of the process. Two such models are examined here and their validities and values considered. We also consider how tests of frequency-dependent selection by predators can best be carried out and what other types of experiment and observation may be relevant.

THE BASIC MODEL

Suppose that in a population of prey two forms are available for a predator, their numbers (relative or absolute) being A_1 and A_2. Suppose further that the probabilities that an individual of either is the first to be taken by the predator are simply related to these frequencies:

$$P_1 = VA_1/(VA_1 + A_2), \quad P_2 = A_2/(VA_1 + A_2)$$

The coefficient V measures the selectivity for the first form compared with the second. It is mathematically equivalent to the 'hunting time' of Tinbergen (1960).

Suppose that eaten individuals are replaced, so that the frequencies are restored to A_1 and A_2, and that the behaviour of the predator is uninfluenced by experience. The probabilities of each of the two forms being taken remain constant at P_1 and P_2 for the second and subsequent acts of predation. After a number of such acts, in which e_1 and e_2 of the two forms have been eaten, then V may be estimated by the cross-product ratio A_2e_1/A_1e_2.

Suppose again that prey are replaced as they are eaten, so that A_1 and A_2 remain constant, but that with experience the predator modifies its behaviour. In this case, if its first choice is form 1, then the probability of taking an individual of form 1 as its second choice is greater than P_1: if its first choice is form 2, then the probability of taking an individual of form 1 as its second choice is less than P_1. Whatever the function relating these probabilities to P_1 and whatever the effect of further experience, the expected value of the cross-product ratio, A_2e_1/A_1e_2, will depart from V in a particular way: if P_1 is greater than 0·5, then there will tend to be disproportionate predation on form 1 and the cross-product ratio will be more than V; if P_1 is less than 0·5 then the reverse will be true, while if $P_1 = 0·5$ then the cross-product ratio will be equal to V. Of course, in particular trials when P_1 is greater than 0·5 an individual of form 2 may be eaten first and as a result there may subsequently be disproportionate predation on form 2. Thus although the expected relationship of the cross-product ratio to V can be described, there is a high variance associated with this expectation. In general, the stronger the effects of previous experience on the predator's behaviour, the more marked will be the departure of the cross-product ratio from V but the greater will be the variance of this departure.

It is not known exactly how selective behaviour is modified by experience. If the two forms of prey represent equal rewards, it is reasonable to suppose that the modification will be the same for each. Thus, if we write $A_2 e_1 / A_1 e_2 = Y = f(A_1 A_2)$, we may also write $A_1 e_2 / A_2 e_1 = 1/Y = f(A_2/A_1)$. Many functions might satisfy these biological constraints and fit available data. The simplest is:

$$e_1/e_2 = (VA_1/A_2)^b, \quad b > 1 \qquad (1)$$

or

$$\frac{e_1}{e_1 + e_2} = \frac{(VA_1)^b}{(VA_1)^b + A_2^b} = \frac{1}{1 + (A_2/VA_1)^b} \qquad (2)$$

(Elton & Greenwood 1970; writing e_1 and e_2 for the N_1 and N_2 of the earlier paper and A_1 and A_2 for D_1 and D_2).

The model is easily generalized to more than two forms of prey:

$$e_i \Big/ \sum_{j=1}^{m} e_j = (V_i A_i)^b \Big/ \sum_{j=1}^{m} (V_j A_j)^b, \quad i = 1, \ 2 \ldots m. \qquad (3)$$

The estimation of V and b is considered in Appendix 1.

The value of b is a measure of the degree of frequency-dependence of the selection. As such it is likely to vary between different predators and different prey. It may also differ between experiments. Thus an experiment in which few prey are eaten may allow less learning than one in which more are eaten, so that the b value will be smaller even if the strength and form of the learning process are the same; when many prey have been eaten, the learning of the apparently rarer form may tend to catch up with the learning of the apparently commoner form, so reducing b; the rate of forgetting may be higher when the rate of predation is lower. If the frequency dependence follows from attempts to optimize foraging, the density of the food and rate of predation may change the optimum strategy and thus the animal's behaviour (S. F. Hubbard & J. J. D. Greenwood, un-published). Notwithstanding these points, a value of b different from unity is a clear indication of frequency-dependence and values obtained in similar experiments may be compared.

When eaten prey are not replaced, so that the relative numbers available to the preda-tor change, it is even more difficult to predict the magnitude of the departure of e_1/e_2 from A_1/A_2. The form of the departure will be the same as when prey are replaced but the magnitude will be smaller. Thus a b value greater than unity will still indicate frequency-dependence, but one will be less likely to find a value significantly greater than unity than in an experiment in which prey are replaced.

In some situations, predation is disproportionately heavy on the rarer forms (Horsley et al. 1979). The value of b is then less than unity.

If $b = 1$, then selection is frequency-independent, so that $V = Y$. Thus V can be taken as a measure of frequency-independent selection, mathematically separate from b—though whether selection can be separated biologically into these two components is debatable.

Manly (1973) has criticized the use of this model, as proposed by Elton & Greenwood (1970) on three grounds. Firstly, that 'it was not developed on the basis of some theory of predator behaviour'. However, while it is true that the precise form of the function relating e_1/e_2 to A_1/A_2 is not based on such a theory, the general form is so based, as explained above. Secondly, 'it is by no means obvious how the relationship can be

generalized to the case of three or more types of prey'. In fact, the generalization is simple: what is not fully solved is the problem of estimating the values of b and V's in the generalized form, which is a valid criticism (see Appendix 2). Thirdly, 'the model can only be used for experiments where the relative prey densities are kept constant by the replacement of eaten prey'. This is not true: if selection is frequency-dependent, then one will obtain larger values of b if the relative prey densities are kept constant than if they are allowed to change but the value of b will still be comparable between experiments of similar design.

MANLY'S MODEL

Manly (1973) proposed that in the analysis of frequency-dependent selection experiments, one may estimate his parameter β for each trial and then look for a significant relationship between β and $A_1/(A_1 + A_2)$ by linear regression analysis, assuming the model $\beta = a + cA_1/(A_1 + A_2)$, where c is positive.

This model may be generalized easily for more than two forms and can be used even when the relative prey densities are not kept constant. Unfortunately, the model is unrealistic in several ways.

The statistical model for the estimation of β assumes that its value remains constant throughout the experiment. In the case of frequency-dependence, therefore, the model is that the predator somehow knows the frequencies of the forms of the prey at the start of the experiment and proceeds to prey on them with a correspondingly adjusted β value. If this is so, the predator's selection is presumably an adaptive response to the prey frequencies (S. F. Hubbard & J. J. D. Greenwood, unpublished) and the predator would thus be expected to modify its selection (β) as the prey frequencies changed. If, to take the other extreme possibility, the predator's behaviour is a simple consequence of learning, then β will change as the learning proceeds. It thus seems unlikely that β remains constant during the course of a trial. It is true that Cook & Miller (1977) found that β did not change markedly between days in an experiment in which prey populations were exposed to pairs of quail (*Coturnix c. japonica*) over a series of days, the initial prey frequencies being restored at the start of each. However, all this need mean is that the birds were behaving in a similar way each day, starting with the same β and modifying it in the same way.

Our second criticism of Manly's model is that it may sometimes predict β values outside the range 0–1 within the domain 0–1 of $A_1/(A_1 + A_2)$, e.g. for the data of Fullick & Greenwood (1979). Such values are clearly impossible.

FITTING THE MODELS TO DATA

Table 1 shows estimates of b and V for all the sets of data presently to hand. The log-log regression and non-linear least squares methods (see Appendix 1) give similar estimates in the majority of cases and, just as there are no *a priori* grounds for favouring one rather than the other, there seem no *a posteriori* grounds either.

None of the data sets show systematic departures from the fitted lines. Where replicate data are available for each value of A_1/A_2, we have carried out goodness-of-fit tests: the fit is excellent in all cases but one—the *Coccinella* trained to *Myzus*, for which $F(3, 25) = 6.4$, $P < 0.01$. These data are, in fact, irregular and accord with no simple model of selection, as Murdoch & Marks (1973) noted.

TABLE 1. Estimates of b and V, with lower (LCL) and upper (UCL) 95% confidence limits, for various sets of data. The non-linear least squares estimates are given first, the log-log regression second. * indicates less than six replicates/frequency at one or two frequencies; brackets indicate that the original data were based on this number of replicates but that only means were available for this analysis. We have omitted experiments in which the number of frequencies tested was less than three and those in which total prey density was allowed to vary more than three-fold. In most experiments only one predator was used in each trial but two quails and several whelks were used in each trial. Note that in the third of Murdoch's experiments, b was not significantly different from zero, so confidence limits for V cannot be calculated in the log-log regression

Predator	Prey	No. of frequencies tested	No. of trials/ frequency	b LCL		UCL	V LCL		UCL	Authors
Bluegill (*Lepomis*)	Larvae of 'midges' and 'mosquitos'	5	(10)	0·52	0·68	0·84	0·72	0·89	1·10	R. C. Reed (in Murdoch &
				0·55	0·68	0·81	0·73	0·88	1·06	Oaten 1975)
Whelk (*Acanthina*)	Mussels (*Mytilus*) and barnacles (*Balanus*)	5	5	−0·07	0·45	0·97	0·65	4·26	25·50	Murdoch (1969)
				0·04	0·49	0·95	1·37	3·98	1000	
Whelk (*Thais*)	Mussels (*Mytilus* and *Septifer*)	3	(6)	0·22	0·60	0·98	0·00	0·02	0·09	Murdoch (1969)
				0·05	0·56	1·07	0·00	0·01	0·14	
Whelk (*Thais*)	Mussels (two *Mytilus* spp.)	3	(5)	−0·49	1·16	2·81	1·40	4·13	12·20	Murdoch (1969)
				−0·95	0·96	2·87	−	4·51	−	
Whelk (*Thais*)	Mussels (two *Mytilus* spp.)	5	3	0·57	1·02	1·47	5·04	9·13	16·50	Murdoch (1969)
				0·94	1·03	1·12	7·17	9·00	11·80	
Ladybird (*Coccinella*) trained to *Myzus*	Aphids (*Aphis* and *Acyrthosiphon*)	5	6*	0·76	0·94	1·12	0·86	1·06	1·31	Murdoch & Marks (1973)
				0·91	1·05	1·19	1·02	1·26	1·55	
Ladybird (*Coccinella*) trained to *Acyrthosiphon*	Aphids (*Aphis* and *Acyrthosiphon*)	5	6	0·55	0·83	1·11	0·50	0·75	1·13	Murdoch & Marks (1973)
				0·56	0·88	1·21	0·57	1·04	1·93	
Ladybird (*Coccinella*) trained to *Aphis*	Aphids (*Aphis* and *Acyrthosiphon*)	5	6*	0·80	0·94	1·08	0·65	0·78	0·94	Murdoch & Marks (1973)
				0·90	1·00	1·10	0·69	0·80	0·93	
Starfish (*Pisaster*)	Whelks (*Acanthina*) and snails (*Tegula*)	4	(3)	0·43	1·03	1·63	0·63	1·12	1·99	Landenberger (1968)
				0·51	1·11	1·71	0·62	1·17	2·54	
Quail (*Coturnix*)	Artificial (two colours)	7	10	1·22	1·40	1·58	1·14	1·26	1·39	Manly, Miller & Cook
				1·25	1·35	1·45	1·18	1·31	1·44	(1972)
Rudd (*Scardinius*)	Waterbug (*Sigara* of two colours)	4	1	0·63	1·40	2·17	1·47	2·12	3·05	Popham (1941)
				1·10	1·44	1·78	1·61	2·20	3·20	
Ciliate (*Stentor*)	Ciliates (*Euglena* and *Chlamydomonas*)	7	(8–12)	1·12	1·70	2·38	1·37	1·80	2·37	D. J. Rapport (in Murdoch
				0·84	1·33	1·82	1·50	2·38	4·77	& Oaten, 1975)
Chick (*Gallus*)	Artificial (two colours)	16	5	1·52	1·70	1·88	1·75	1·87	1·99	Fullick & Greenwood
				1·51	1·59	1·67	1·75	1·88	2·02	(1979)
Waterbug (*Notonecta*)	Mayfly larvae (*Chloëon*) and waterlice (*Asellus*)	5	5	1·44	1·98	2·52	0·98	1·10	1·23	Lawton, Beddington
				1·60	1·95	2·31	0·83	0·98	1·16	& Bonser (1974)

Figure 1 shows the fit of the model to four of these sets of data, both the non-linear least squares fit in terms of proportions of the prey types and the log-log regression fit in terms of logs of the ratios of the prey types. For *Notonecta*, $b > 1$, $V = 1$; for *Coturnix* and *Stentor* $b > 1$, $V > 1$; for *Lepomis* $b < 1$, $V \simeq 1$. The *Stentor* data are one of those sets in which the agreement between the two methods of fitting the model is not close. Figure 1 shows why: the data are irregular and the experimental proportions of prey offered are very asymmetrically distributed about the point where $e_1/e_2 = A_1/A_2$.

Manly (1973) has successfully fitted his model to some of the same data. We have no doubt that it would fit most of the rest, since his model is similar to ours except at extreme frequencies, when it predicts less extreme selection than does ours. Fortunately, data are now available that cover a sufficient range of relative frequencies for the difference between the models to be tested—those of Fullick & Greenwood (1979). For this data set, our model is an adequate fit, whereas that of Manly is not. The Manly values do not show a straight-line relationship to the values of $A_1/(A_1 + A_2)$ and, furthermore, the best straight-line fit to the data predict β values greater than unity for $A_1/(A_1 + A_2) > 0.94$.

A variant of the Manly model is that the logarithm of α, a selective coefficient related to β, is linearly related to $A_1/(A_1 + A_2)$ (Cook & Miller 1977). This implies that β is logistically related to $A_1/(A_1 + A_2)$ (Manly, Miller & Cook 1972). However, this variant is no better fit to the data in question than the basic model (Fullick & Greenwood 1979).

Apparently simpler methods of analysing the data may be misleading because they do not use all the information in it. Thus Murdoch (1969) assessed 'preference' by measuring the ratio e_1/e_2 when $A_1 = A_2$, thus ignoring the information about the frequency independent component of selection contained in trials when $A_1 \neq A_2$. This led him to say that *Acanthina* had only a weak preference for barnacles over mussels but that *Thais* had a strong preference for *Mytilus edulis* over *M. californianus*, though the total data indicate no difference between them in the value of V (Table 1).

NOTES ON EXPERIMENTAL DESIGN

When designing experiments of this type it is helpful to have some idea of the value of A_1/A_2 for which $e_1/e_2 = A_1/A_2$. Statistical efficiency is likely to follow when the experimental values of $\log(A_1/A_2)$ are symmetrically distributed about this point. As with all regression problems, there are advantages to having an even spread of values of A_1/A_2, to having a wide range of such values, and to having replicates at each value. The best design will vary according to the values of b and V and according to which of these it is more important to estimate accurately, so no general advice can be given except that all three desiderata need to be considered. It is clearly best if the frequencies of the various forms of prey are kept constant within each trial. Though Popham (1941, 1943) did this, it is rarely possible without disturbing the experimental animals. A useful compromise is to restore the original frequencies at intervals during the experiment, either when set numbers of prey have been eaten or at set times (Landenberger 1968, Murdoch 1969, Manly, Miller & Cook 1972, Cook & Miller 1977). Another technique is to terminate each trial before a large proportion of the prey has been eaten (Fullick & Greenwood 1979).

For b values to be comparable between experiments, it is important to hold as many factors constant as possible. This is especially true for the length of the experiment and the number of prey eaten, since these will affect learning. Of course, it will rarely be

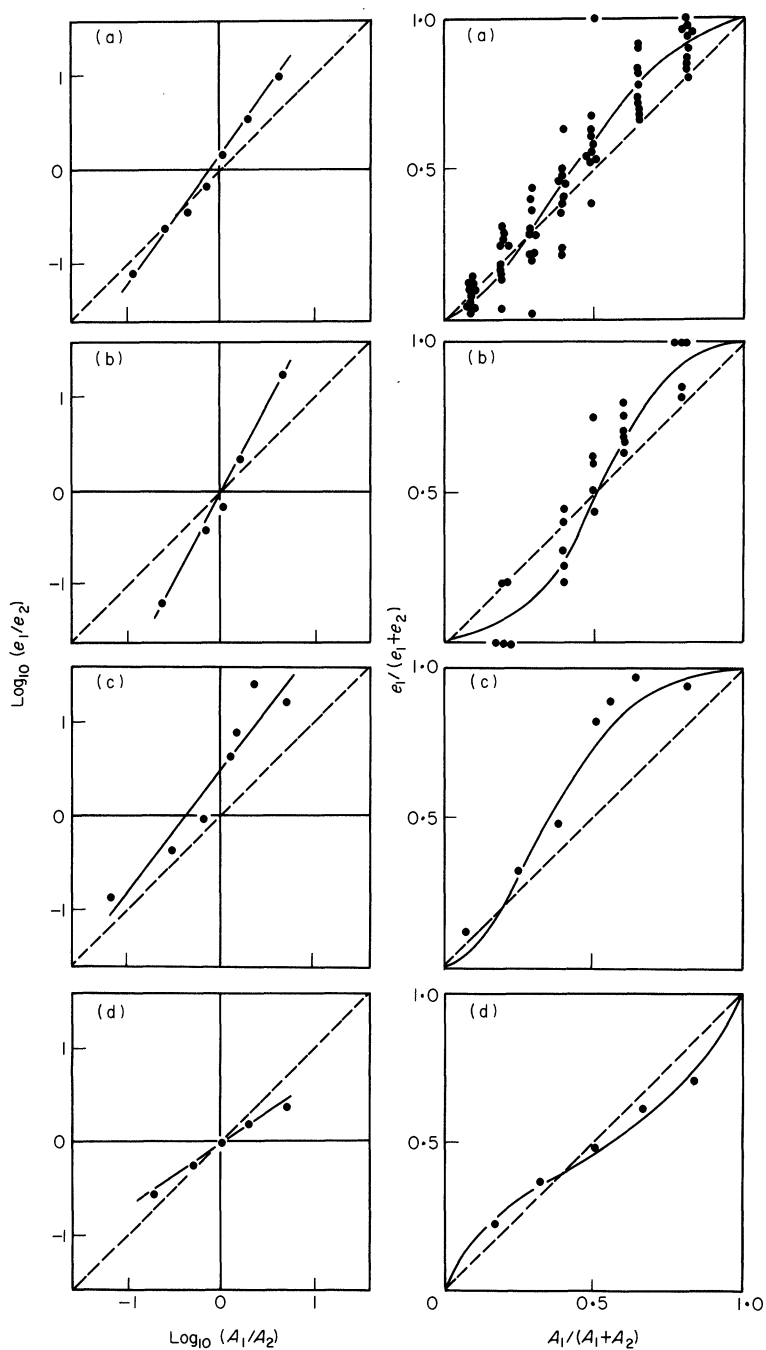FIG. 1. Relationship of relative numbers of two forms eaten to relative numbers available for (a) *Coturnix* feeding on artificial food, (b) *Notonecta* feeding on *Chloëon* and *Asellus*, (c) *Stentor* feeding on *Euglena* and *Chlamydomonas*, and (d) *Lepomis* feeding on midges and mosquitos. A_1 and A_2 are the numbers of the two forms of food available e_1 and e_2 the numbers eaten.

possible to stabilize both simultaneously: the choice of which to stabilize will usually be determined by the experimental situation.

Further experiments are needed on a diversity of predators and prey, to establish how widespread and how strong frequency-dependent selection is. We also need to know more about the effects on the strength of the frequency-dependence of overall density of the prey, of rate of predation in time, and of total number of prey consumed. These may all be investigated using the method discussed in this paper.

When performing experiments with predators which are difficult to obtain or keep, there is a temptation to use the same few individual predators in a series of trials or experiments (e.g. Croze 1970; Mueller 1971). Since memory and a high variance between individuals are central to this topic, this is generally unsatisfactory.

OTHER RELEVANT TYPES OF EXPERIMENTS AND OBSERVATIONS

Allen (1972, 1976) exposed two forms of prey to predators in the ratios 9:1 for some predators and 1:9 for others. He then reversed the ratios presented to each group of predators. This allowed direct comparison of the choice of each group at each ratio, testable by χ^2 in the usual way. In our method, or that of Manly, the heterogeneity between predators is not overcome by making comparisons 'within' predators but it is included in the error variance associated with the fitted curve. Using Allen's technique, there is no valid way of combining data from the several trials, because of the heterogeneity between them. To obtain an overall view of the pattern of the data, Allen was reduced to counting the number of trials in which the selection changed in the direction expected on the frequency-dependence hypothesis. Since his results were clear, this was satisfactory but it is clearly a method that loses much of the information in the original data.

Experiments in which predators are 'trained' on monomorphic prey before being presented with the two forms have generally indicated that training can be effective (Allen & Clarke 1968; Landenberger 1968; Murdoch 1969; P. Oates in Cook 1971; Soane & Clarke 1973; Allen 1974). Such experiments are relevant to situations in which the prey frequencies are spatially or temporally heterogeneous, in that the predator may not respond immediately to changes in prey frequencies as it moves from place to place or as seasonal changes occur. This may lead to frequency-dependent selection in the prey population even when the predator's behaviour is not frequency-dependent. However, such experiments do not demonstrate that either the predator's behaviour or the selection in the prey population is frequency-dependent in the absence of the imposed temporal heterogeneity of prey frequencies.

If a prey population is exposed to a number of predators and one finds a higher variance of their choices than one would expect if their preferences were identical, then one must assume that their preferences are not identical. Murton (1971) found this for wood pigeons (*Columba palumbus*) eating seeds and concluded that the difference in preferences was an effect of learning during the experiment. However, the preferences could be predetermined, so such an experiment is not a satisfactory test of learning, nor does it tell us whether or not selection is likely to be frequency-dependent.

Following Tinbergen (1960), various workers have looked for evidence of learning and frequency-dependent selection from the natural diets of wild predators. Such evidence can usually be interpreted both in terms of learning what prey looks like and in terms of learning where prey is to be found (Royama 1970). While both processes may be

ecologically and genetically important, their effects may be different, so they need to be distinguished. Since the predators can learn both appearance and location of prey (Alcock 1973), observations on natural predation must be detailed if one is to determine which is the more important in any particular case. Such detailed field-studies are difficult, though a number of relevant ones have now been made (Goss-Custard 1970a, b, 1977a, b, c; Baker 1973, 1974; Smith 1974a, b).

To describe frequency-dependent selective behaviour fully, it will be necessary to know more about the learning process. The relevance of much of the standard work on learning is not obvious. Simple experiments in which the sequence of choices from a mixed prey population is observed (e.g. Dawkins 1971) should allow the empirical model suggested here to be replaced by an analytical model. We are conducting both experimental and theoretical work to this end.

·ACKNOWLEDGMENTS

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APPENDIX 1

PARAMETER ESTIMATION: TWO PREY TYPES

Estimation

The logarithmic transformation of eqn 1 is

$$\log e = b \log V + b \log A,$$

where $e = e_1/e_2$ and $A = A_1/A_2$.

Thus normal linear regression methods using values of $y = \log e$ and $x = \log A$, derived from a set of n trials, will give estimates of the regression parameters:

$$\alpha = b \log V \quad \text{and} \quad \beta = b.$$

(Note that this is not the parameter α used by Manly 1973, 1974.)

The estimates of these parameters and of the residual variance are:

$$\hat{\alpha} = \bar{y} - \hat{\beta}\bar{x}$$
$$\hat{\beta} = S_{xy}/S_{xx}$$

and

$$s^2 = (S_{yy} - S_{xy}^2/S_{xx})/(n - 2)$$

where S_{yy}, S_{xy}, and S_{xx} are the usual corrected sums of squares and sum of products.

The confidence intervals for α and β are

$$\hat{\alpha} \pm t\sqrt{s^2(1/n + \bar{x}^2/S_{xx})}$$
$$\hat{\beta} \pm t\sqrt{s^2/S_{xx}}$$

where t is the required percentage point of Student's t distribution with $n - 2$ degrees of freedom.

The estimate and confidence intervals of the model parameter b are, of course, those of β. The ratio $\hat{\alpha}/\hat{\beta}$ estimates $\log V$, from which V may be estimated by taking antilogs.

A confidence interval for $\log V$ can be obtained if $\hat{\beta}$ is significantly different from zero. (If it is not then either the data are not worth analysing or one is witnessing a most peculiar type of behaviour.) The interval is the range (θ_1, θ_2) where θ_1 and θ_2 are the two solutions of the quadratic equation

$$\theta^2(\hat{\beta}^2 - t^2 \cdot s^2/S_{xx}) - 2\theta(\hat{\alpha} \cdot \hat{\beta} + t^2 \cdot s^2\bar{x}/S_{xx}) + \hat{\alpha}^2 - t^2 \cdot s^2(1/n + \bar{x}^2/S_{xx}) = 0.$$

The confidence limits for V are the antilogs of these. Note that they are asymmetrical. The distribution of V is, indeed, not Normal, which has implications for hypothesis testing.

Approximate standard errors for b and $\log V$ may be obtained from

$$\text{S.E.} (\hat{b}) = s/\sqrt{S_{xx}}$$
$$\text{S.E.} (\log \hat{V}) = (s/\hat{\beta})\sqrt{(1/n + \bar{y}^2/\hat{\beta}^2 S_{xx})}.$$

The log-log regression method cannot be used if no individuals of one prey type are eaten in any trial, since this gives values for $\log e$ of plus or minus infinity. One can often overcome this problem by combining all the replicate trials for each relative prey density available. More generally, however, it seems unwise to use this method if any of the trials have very large or very small values of e: these give large absolute values of $\log e$, so that these trials are given undue weight in the analysis.

In such circumstances, we suggest as an alternative that a relationship be fitted between A, the ratio of frequencies offered, and p, the proportion of the first type taken:

$$p = e_1/(e_1 + e_2).$$

Since the range of possible sample values of p is between 0 and 1, the problem of values tending to infinity is eliminated. However, the relationship of p to A is not linear under our model:

$$p = (VA)^b/[1 + (VA)^b]$$

and so the parameters V and b must be estimated from the sample data $\{(p_i, A_i), i = 1, \ldots, n\}$ by non-linear least squares techniques. This may be done iteratively using a Taylor series linear approximation to the function (Draper & Smith 1966).

The method starts by using initial approximate values $V^{(0)}$ and $b^{(0)}$, which are estimated in practice from the log-log regression or, if this has not been done, from a visual fit to the points. A Taylor series expansion of p then gives:

$$p(V, b) - p(V^{(0)}, b^{(0)}) = (V - V^{(0)})\, \partial p(V^{(0)}, b^{(0)})/\partial V + (b - b^{(0)})\, \partial p(V^{(0)}, b^{(0)})/\partial b + R,$$

where R represents higher order terms in $V - V^{(0)}$ and $b - b^{(0)}$. If these terms are ignored, this equation describes a linear relationship between the difference of the p values using the true and estimated parameters and the differential coefficient terms. Thus the parameters $\psi = V - V^{(0)}$ and $\phi = b - b^{(0)}$ can be estimated by linear regression using sample values of these variables. If we define

$$p_i^{(0)} = (V^{(0)} A_i)^{b^{(0)}} / [1 + (V^{(0)} A_i)^{b^{(0)}}]$$

then the dependent variable in this regression is

$$Z_i = p_i - p_i^{(o)}.$$

The independent variables are calculated from the differential coefficients:

$$w_i = b^{(0)} p_i^{(0)} (1 - p_i^{(0)}) / V^{(0)}$$

and

$$x_i = \ln(V^{(0)} A_i) p_i^{(0)} (1 - p_i^{(0)}),$$

and the coefficients ψ and ϕ are estimated by linear multiple regression of z on w and x, using the model

$$z = \psi w + \phi x.$$

The estimates ψ and ϕ resulting from this calculation are used to give improved estimates of V and b:

$$V^{(1)} = V^{(0)} + \hat{\psi}$$

and

$$b^{(1)} = b^{(0)} + \hat{\phi}$$

and the cycle of estimation is repeated until no appreciable improvement in fit can be obtained. Approximate standard errors for the final estimates $\hat{V}$ and $\hat{b}$ are given by the standard errors of the estimates $\hat{\psi}$ and $\hat{\phi}$ from the final round of multiple regression.

The standard error of log $\hat{V}$, more useful in hypothesis testing, is obtained from

$$\text{S.E. (log } \hat{V}) = \text{S.E. } (\hat{V})/2\cdot3026\hat{V}$$

omitting the factor $2\cdot3026$ if natural logarithms are being used rather than logarithms to base 10.

From experiments in which replicate trials have been carried out at each relative frequency (Fig. 1 and Fullick & Greenwood 1979), it is clear that the variance of p about the regression line is smaller at extreme frequencies than for those in the middle of the range. Thus the non-linear least squares method gives too little weight to these data, just as the log-log regression gives too much. However, the two methods provide

estimates of b and V that are usually very close (Table 1), indicating that there is no need to adopt more precise methods, such as weighted regression. Only if the fit of the model to the data is rather poor—as for the *Stentor* data (Fig. 1)— is there a large difference in the two sets of parameter estimates. Since it is the simpler of the two methods, we recommend that log-log regression be used where possible.

We previously suggested (Elton & Greenwood 1970) that logit analysis might be used to estimate b and V. This involves fitting the model by an iterative maximum-likelihood method which depends on the assumption that the data points are binomially distributed about the fitted line. In fact, if positive frequency-dependent selection is taking place the variances of the replicate trials at each frequency of presentation are much larger than binomial expectation (Murdoch & Oaten 1975). We cannot, therefore continue to recommend this method since it will underestimate the standard errors of the estimates, even if the estimates themselves are reasonably accurate.

Hypothesis testing

Whichever method is used, the departure of $\hat{b}$ from unity may be tested by the approximate t-test:

$$t = (\hat{b} - 1)/\text{S.E.} (\hat{b})$$

with $n - 2$ d.f.

A similar test may be applied to $\hat{V}$. However, the distribution of $\hat{V}$ is far from normal and we have found cases in which an estimate is very significantly different from unity but an estimate of its reciprocal, obtaining by interchanging the two prey types in the formulae, is not. Because of the lack of normality and the asymmetry, we recommend testing instead the departure of the estimate of log $\hat{V}$ from zero.

In the log-log regression analysis, $\alpha = b \log V$, so we test

$$t = \hat{\alpha}/\text{S.E.} (\hat{\alpha}).$$

The non-linear least squares model can be reparameterized to estimate b and $\log V$, rather than b and V, or one may estimate $\log V$ from $\hat{V}$ and its standard error from

$$\text{S.E.} (\log \hat{V}) = \text{S.E.} (\hat{V})/2 \cdot 3026 \hat{V}$$

omitting the factor $2 \cdot 3026$ if natural logarithms are being used rather than logarithms to base 10.

If two experimental estimates of b are available, the difference between them may be tested by using

$$z = (\hat{b}_1 - \hat{b}_2)/\sqrt{[\text{S.E.} (\hat{b}_1)^2 + \text{S.E.} (\hat{b}_2)^2]}$$

which will usually approximate to a standard normal variate provided that the number of trials is reasonably large in each experiment. Twenty is probably the minimum number in each experiment for this assumption to be safe. A more accurate method, recommended for smaller numbers of trials, is to test z against Student's t with

$$(n_1 - 2)(n_2 - 2)[\text{S.E.} (b_1)^2 + \text{S.E.} (b_2)^2]^2/[(n_1 - 2) \text{S.E.} (b_1)^4 + (n_2 - 2) \text{S.E.} (b_2)^4]$$

degrees of freedom (Welch 1949).

Two values of V may be compared similarly, though again it is probably safer to work with estimates of log V.

APPENDIX 2
PARAMETER ESTIMATION: MORE THAN TWO PREY TYPES

Method

Generalizations of the log-log regression and non-linear least squares methods for more than two prey types are not available. It is, however, easy to generalize the maximum likelihood method and we give details of the method and a worked example here. The parameter estimates are likely to be reasonably accurate if several replicate trials are carried out at each of several relative frequencies but the estimated standard errors will be less reliable.

Suppose that m morphs are presented in each of k different presentations. Eqn 3 may be written

$$p_{ij} = \frac{e_{ij}}{\sum\limits_{j=1}^{m} e_{ij}} = \frac{(V_j A_{ij})^b}{\sum\limits_{j=1}^{m} (V_j A_{ij})^b} \quad i = 1, 2, \ldots, k; j = 1, 2, \ldots, m$$

$$= \frac{\exp(\alpha_j + b \cdot_j x_{ij})}{\sum\limits_{j=1}^{m} \exp(\alpha_j + b \cdot x_{ij})} \quad \text{where } \alpha_j = b \ln V_j, \text{ and } x_{ij} = \ln A_{ij}. \tag{4}$$

From standard multinomial theory,

$$\text{likelihood} = \prod_{i=1}^{k} \frac{g_i!}{e_{i1}! \ldots e_{im}!} \prod_{j=1}^{m} p_{ij}e_{ij}$$

where $\{e_{ij}\}$ now represent the observed numbers in the experiment and $g_i = \sum_{j=1}^{m} e_{ij}$
This gives a log likelihood of

$$L = \text{constant} + \sum_{i=1}^{k} \sum_{j=1}^{m} e_{ij} \ln p_{ij}.$$

Values of V may, of course, only be measured relative to each other. Therefore we arbitrarily set $V_1 = 1$ ($\alpha_1 = 0$) and require to estimate the m parameters $\alpha_2, \ldots, \alpha_m, b$. To do this, one must solve the m simultaneous equations

$$\frac{\partial L}{\partial \alpha_j} = 0, \quad j = 2, \ldots, m$$

$$\frac{\partial L}{\partial b} = 0.$$

It can be shown, from eqn 4, that

$$\frac{\partial p_{ij}}{\partial \alpha_j} = p_{ij}(1 - p_{ij}), \quad j = 2, \ldots, m$$

$$\frac{\partial p_{ij}}{\partial \alpha_1} = -p_{i1}p_{ij}, \quad j \neq l, j = 1, \ldots, m, l = 2, \ldots, m$$

$$\frac{\partial p_{ij}}{\partial b} = p_{ij}\left(x_{ij} - \sum_{l=1}^{k} x_{il}p_{il}\right), \quad j = 1, \ldots, m.$$

Thus

$$\frac{\partial L}{\partial \alpha_j} = \sum_{i=1}^{k} \sum_{l=1}^{m} \frac{e_{il}}{p_{il}} \frac{\partial p_{il}}{\partial \alpha_j}$$

$$= \sum_{i=1}^{k} e_{ij} - \sum_{i=1}^{k} g_i p_{ij}, \quad j = 2,\ldots,m,$$

and

$$\frac{\partial L}{\partial b} = \sum_{i=1}^{k} \sum_{j=1}^{m} e_{ij} x_{ij} - \sum_{i=1}^{k} g_i \left(\sum_{j=1}^{m} x_{ij} p_{ij} \right).$$

So one must solve:

$$\left.\begin{aligned} \sum_{i=1}^{k} g_i p_{ij} &= \sum_{i=1}^{k} e_{ij} \quad j = 2,\ldots m \\ \sum_{i=1}^{k} g_i \left(\sum_{j=1}^{m} x_{ij} p_{ij} \right) &= \sum_{i=1}^{k} \sum_{j=1}^{m} e_{ij} x_{ij} \end{aligned}\right\} \text{for } \alpha_2,\ldots,\alpha_m, b.$$

This may be done iteratively, using the Newton-Raphson method

$$\theta^{(t+1)} = \theta^{(t)} - \mathbf{D}^{-1}.\mathbf{C}$$

where $\theta^{(t)}$ is the vector of estimated parameters after the tth iteration, $\mathbf{C}$ is the vector of first derivatives of the likelihood, evaluated at $\theta^{(t)}$, and $\mathbf{D}$ is the vector of second derivatives of the likelihood, also evaluated at $\theta^{(t)}$. (Note that D_0, $\mathbf{D}$ evaluated at $\theta^{(0)}$ may be used throughout, to avoid inverting the $\mathbf{D}$ matrix at each iteration, but that this will result in slower convergence of the iterations.)

The second derivatives are:

$$\frac{\partial^2 L}{\partial \alpha_j^2} = \sum_{i=1}^{k} g_i p_{ij}(1 - p_{ij})$$

$$\frac{\partial^2 L}{\partial \alpha_j \, \partial \alpha_l} = \sum_{j=1}^{k} g_i p_{ij} p_{il} \quad l \neq j,$$

$$\frac{\partial^2 L}{\partial \alpha_j \, \partial b} = \sum_{i=1}^{k} \left(g_i p_{ij} \sum_{l=1}^{m} x_{il} p_{il} \right) - \sum_{i=1}^{k} g_i x_{ij} p_{ij},$$

$$\frac{\partial^2 L}{\partial b^2} = \sum_{i=1}^{k} g_i \left\{ \left(\sum_{j=1}^{m} x_{ij} p_{ij} \right)^2 - \sum_{l=1}^{m} x_{ij}^2 p_{ij} \right\}.$$

When the final estimates $\theta^{(t)}$ are obtained, estimates of the standard errors of the estimates can be calculated from the square roots of the diagonal elements of $-\mathbf{D}^{-1}$ evaluated at $\theta^{(t)}$.

Example

To illustrate this method, we use the data of Popham (1943). Three colours of the aquatic hemipteran *Sigara distincta* (Fieb.) were exposed at six sets of relative frequencies to predation by rudd (*Scardinius eryophthalmus* L.). Eaten prey were replaced as they

TABLE 2 Data from Popham (1943), Table 3

Trial no.	Logs of relative nos. available			Nos. Eaten			Total
i	x_{i1}	x_{i2}	x_{i3}	e_{i1}	e_{i2}	e_{i3}	g_i
1	0	0	0	105	67	28	200
2	0	0·693	0	56	132	12	200
3	0·693	0	0	144	40	16	200
4	0	0	0·693	55	25	108	188
5	0	0	1·099	46	23	131	200
6	0	0·693	1·099	26	62	105	196

were removed. The data are shown in Table 2. Notice that we are interested in relative values of A_{ij}, so it is sensible to set one value of $x(= \log A)$ in each row to zero.

From these data:

$$\sum_i e_{i2} = 349; \sum_i e_{i3} = 400; \sum_i \sum_j e_{ij}x_{ij} = 568\cdot416.$$

If we take the simplest hypothesis for our initial estimates, that there is no frequency-dependence and all prey are equally likely to be eaten, $b = 1$ and $\alpha_2 = \alpha_3 = 0$. From these assumed values and the data we may calculate

$$\{g_i p_{ij}\} = \begin{bmatrix} 66\cdot67 & 66\cdot67 & 66\cdot67 \\ 50 & 100 & 50 \\ 100 & 50 & 50 \\ 47 & 77 & 94 \\ 40 & 40 & 120 \\ 32\cdot67 & 65\cdot33 & 98 \end{bmatrix}.$$

From this

$$\sum_i g_i p_{i2} = 369; \sum_i g_i p_{i3} = 478\cdot67; \sum_i \sum_j x_{ij} g_i p_{ij} = 488\cdot568,$$

so that the first derivatives are -20, $-78\cdot67$, $79\cdot85$.

The second derivatives may also be calculated:

$$\partial^2 L/\partial\alpha_2^2 = -\sum_i g_i p_{i2}(1 - p_{i2}) = -242\cdot748$$

$$\partial^2 L/\partial\alpha_3^2 = -\sum_i g_i p_{i3}(1 - p_{i3}) = -263\cdot444$$

$$\partial^2 L/\partial\alpha_2 \, \partial\alpha_3 = \sum_i g_i p_{i2} p_{i3} = 139\cdot889$$

$$\partial^2 L/\partial\alpha_2 \, \partial b = \sum_i (g_i p_{i2} x_{i3} p_{i3}) - \sum_i g_i x_{i2} p_{i2} = 31\cdot025$$

$$\partial^2 L/\partial\alpha_3 \, \partial b = \sum_i (g_i p_{i3} x_{i2} p_{i2}) - \sum_i g_i x_{i3} p_{i3} = -81\cdot843$$

$$\partial^2 L/\partial b^2 = \sum_i g_i \left\{ \left(\sum_i x_{ij} p_{ij}\right)^2 - \sum_j x_i^2 p_{ij} \right\} = -158\cdot876.$$

Hence

$$\mathbf{D} = \begin{bmatrix} -242\cdot748 & 139\cdot889 & 31\cdot025 \\ 139\cdot889 & -263\cdot444 & -81\cdot843 \\ 31\cdot025 & -81\cdot843 & -158\cdot876 \end{bmatrix}.$$

The new set of estimates of the model parameter is then:

$$\begin{bmatrix} \alpha_2 \\ \alpha_3 \\ b \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} - \overset{D^{-1}}{\begin{bmatrix} -20 \\ -78 \cdot 67 \\ 79 \cdot 85 \end{bmatrix}} = \begin{bmatrix} -0 \cdot 43 \\ -0 \cdot 78 \\ 1 \cdot 81 \end{bmatrix}.$$

Further rounds of iteration may then proceed. Three further rounds give values differing only in the third significant figure from those obtained after two, viz.

$$\hat{\alpha}_2 = -0 \cdot 45$$
$$\hat{\alpha}_3 = -0 \cdot 91 \quad \text{with S.E.} \quad \begin{cases} 0 \cdot 079 \\ 0 \cdot 088 \\ 0 \cdot 100. \end{cases}$$
$$b = 1 \cdot 95$$

From these values, if $V_1 = 1$ (arbitrary), then $\hat{V}_2 = 0 \cdot 79$ and $\hat{V}_3 = 0 \cdot 63$.

What reliance can be placed on these estimates? The ratios of $\hat{\alpha}_2$, $\hat{\alpha}_3$, and $b - 1$ to their standard errors are 5·7, 10·3 and 9·5. The 5% value of Student's t for 4 d.f. is 2·8. Thus, even allowing for the problems associated with the use of this method, it seems reasonable to suppose that predation in this experiment was frequency-dependent ($b > 1$) and that the second and third forms of prey were less visible or less preferred than the first ($\alpha_2 < 0$, $\alpha_3 < 0$). If $\hat{\alpha}_2$ and $\hat{\alpha}_3$ are compared in the usual way, $t = 3 \cdot 9$: there are again 4 d.f. since $\hat{\alpha}_2$ and $\hat{\alpha}_3$ come from the same data set. Hence, given the problems associated with the use of the method, the apparently lower V of type 3 than of type 2 may not be reliable.