FREQUENCY RELATED OVIPOSITION BEHAVIOUR AND HOST SEED PREFERENCE OF BRAZIL AND YEMEN STRAINS OF COWPEA WEEVIL (*Callosobruchus maculatus* (F.))

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ABSTRACT

Experiments conducted to analyse the oviposition behaviour and host seed preference of Brazil and Yemen strains of cowpea weevil (*Callosobruchus maculatus (F.)*) revealed that the host seed selection behaviour of these two strains was frequency independent. The apparent host seed preference of these two strains depended on the total seed density available for oviposition. In low seed densities, females tended to lay eggs on less preferred seeds when attractiveness of the preferred seeds declined due to oviposition marker. Host seed preference of Yemen strain corresponded to progeny fitness while in Brazil strain there was no correspondence between the two traits. These findings also indicated the importance of total seed density in analysing the oviposition behaviour of cowpea weevil as seeds bearing eggs become less attractive for further oviposition.

KEY WORDS: ***Callosobruchus maculatus*, Cowpea weevil, Frequency related oviposition, Host seed preference, Progeny fitness, Oviposition marker.**

INTRODUCTION

The southern cowpea weevil, *Callosobruchus maculatus*(F.) is a major pest of stored legumes in the tropics and the subtropics. The individual populations of this weevil show great variation in host seed preference (Wasserman, 1986; Credland, 1990). Such variation of host seed preference in insects may be traced to either of two courses. Firstly, the populations may be genetically different with respect to host plant preference and other bionomic characters. Secondly, this variation may be due to the different environments they have experienced either as adults or juveniles (Prokopy *et al.*, 1982). It has been demonstrated that the food preference of some animals could be altered in favour of a particular food type when the adult females are allowed to feed or oviposit on the same food type for several generations (Wasserman and Futuyama, 1981).

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The phenomenon called **Frequency Related Food Selection** postulates that in a two choice environment, the food selection of an animal could be a function of their relative availability (Murdoch and Oaten, 1975; Amalraj and Das, 1996). Ideally certain animals tend to select more common food type (positive frequency dependence) whereas others may select less common food type (negative frequency dependence) available in a given environment. In frequency independent behaviour the animal maintains a fixed preference and as a result the attack will always be directed towards the preferred food type. The functional basis of frequency independent selection has been described as an adaptation to maximise individual fitness with increased rate of energy gain (Pyke *et al.*, 1977).

Analysis of the frequency related food selection behaviour may be an attractive means of explaining the variation of host seed preference of the population of cowpea weevil, because frequency dependent host seed selection could lead to some behavioural and physiological adaptation to a particular food type with respect to its relative availability.

The purpose of this study is to examine the frequency related host seed selection and host seed preference of Brazil and Yemen strains of *Callosobruchus maculatus* in the context of their oviposition strategies to maximise progeny fitness.

In order to describe the frequency related food selection behaviour and host seed preference, the Greenwood-Elton model was used (Greenwood and Elton, 1979).

MATERIALS AND METHODS

Three stored commodities of legumes, cowpea *(Vigna unguiculata* (L.) Walp), greengram (*Vigna radiata* (L.)Wilczek) and adzuki beans (*Phaseolus angularis* (Willd.) Wight) were used in this study. The seeds were purchased in bulk from local Health Food Suppliers. All the seeds were frozen at -18^0 C for one week to kill any prior infestation by insects and diseases and were equilibrated to experimental conditions for 4-6 weeks before use.

Two strains of *Callosobruchus maculatus*, Brazil(CMB) and *Callosobruchus maculatus* Yemen (CMY), obtained from the Natural Resources Institute, U.K were used in the study. The strains of insects were cultured continuously on cowpea in the controlled temperature and humidity (CTH) room maintained at 30±1°C and 70±5% RH in the Department of Pure and Applied Zoology, University of Reading, U.K.

For obtaining virgin females and males, about 50 adults collected from routine cultures were introduced into glass bottles containing 125 grams of greengram seeds. After 24 hours of introduction the adults were separated by sieving and the seeds with eggs of same age were obtained. The seeds were isolated in glass vials and the beetles that emerged from them were sexed immediately using the characteristics described by Southgate *et al.*, (1957).

Oviposition behaviour of the Brazil and Yemen strains of *C. malculatus* was monitored on three seed mixtures, viz, cowpea- greengram (C/G), cowpea-adzuki (C/A) and greengram-adzuki (G/A). These mixtures were offered in three different ratios, viz, 3:1, 1:1 and 1:3 at three different total seed densities (40, 80, and 160 seeds). Thus at a total seed density of 40 treatments contained seeds in the ratio of 30:10, 20:20, 10:30. Accordingly treatments in other mixtures contained seeds in the ratio of 60:20, 40:40, 20:60 at a total seed density of 80 and in the ratio of 120:40, 80:80, 40:120 at a total seed density of 160. The seed mixtures were offered in Belfast-disposable plastic petri dishes (ART 721). The lid of each petri dish was perforated to enable air circulation. A total of 10-20 replicates were set up in each experiment. A pair of newly emerged beetles was introduced to each petri dish. After 15 days total number of eggs laid on the host seeds was counted.

The model

Greenwood-Elton model is graphically represented in Fig. 1. And its mathematical basis is explained below.

If e_1 and e_2 are the number of eggs laid in each food type and a_1 and a_2 are the number of seeds available for oviposition from the two food types, it can be assumed under the frequency independent selection that

$e_1 = a_1v_1$, $e_2 = a_2 v_2$ where v_1 and v_2 are measures of preference of the two food types offered. Suppose a_1 is preferred to a_2 by some 'V' value so that $V = v_1/ v_2$ and the selection of the food types is related to the frequency of availability of the food types, then

$$p_1 = Va_1/(va_1+a_2)\ , \quad p_2= a_2/(va_1+a_2)$$

where p_1 and p_2 are the probabilities of each food types taken, i.e. $p_1=e_1/e_1+e_2$ and $p_2 = e_2 /e_1+e_2$

When $p_1 > 0.5$, then there will be a disproportionate predation on food type 1 and a_2e_1/a_1e_2 will be more than V. When $p_1 = 0.5$, a_2e_1/a_1e_2 will be equal to unity. when there is a positive frequency dependence, $e_1/e_2 = (Va_1/a_2)^b$ with $b > 1$. Log transformation of this equation is

Log e = b log V + b log a Where, $e=e_1/e_2$ and $a= a_1/a_2$

Let the intercept, when log a=0, be æ , so that æ= b log V. Then, æ/b = log V.

The plot indicates the frequency dependent oviposition by departure of slope (b) from unity, and b value significantly higher than unity indicates positive frequency dependence while the b value significantly lower than unity indicates negative frequency dependence. The V value significantly higher than unity indicates the preference for numerator food type offered in the mixture while V value significantly lower than unity indicates the preference for denominator food substrate. When V is equal to unity the animal shows equal preference for the two food types. This model was modified to describe the frequency related host seed preference of cowpea weevil by plotting the ratio of eggs laid against the seeds offered·

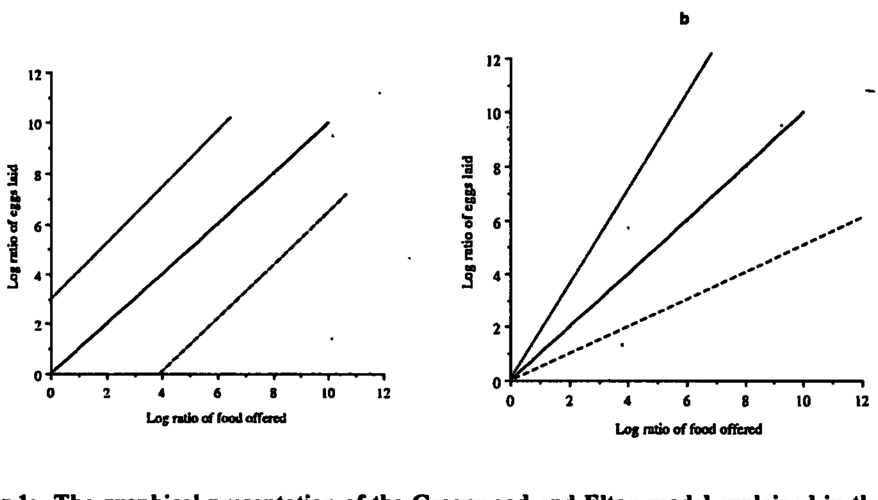

Fig.1: The graphical presentation of the Greenwood and Elton model explained in the text. a). The solid line indicates equal preference ; dotted line and dashed line represent the preference for denominator and numerator food substrates respectively. b) Solid line indicates expected ratios in case of frequency independence. Dotted and dashed lines illustrate the general form of positive frequency dependence and negative frequency dependence respectively.

RESULTS

The Greenwood-Elton model used in this study has been formulated to describe the food selection behaviour of higher animals with little reference to insects. However, the model provides a good fit to the data (*e.g.* Figs. 2 and 3).The estimates of parameters b (index of frequency related host seed selection) and V (index of host seed preference) of CMB and CMY are given in Tables 1 and 2 respectively.

In all cases the host seed selection behaviour is frequency independent as b was not significantly different from unity (Tables1 and 2). The results obtained at total seed densities of 40, 80 and 160 are plotted in Figs. 2 and 3 in the same way as in Fig. 1. When the total seeds in each treatment was limited to 40 in C/G and C/A mixtures with CMB as the test insect, the V values obtained were 3.12 and 5.81 respectively. These V values being significantly higher than unity indicate that CMB prefers cowpea for oviposition. However, CMB changed its preference towards greengram (V=0.37) when the total seed density in C/G mixture increased to 160. When the total seed density in the C/A mixture increased to 80, CMB changed its preference towards adzuki (V= 0.77). However, on further increase of the total seed density in the C/A mixture, CMB changed its preference towards cowpea (V= 3.43).

At a total seed density of 40, CMY showed equal preference for the two available seed types in C/G (V=0.96) and G/A (V=1.21) mixtures (Table. 2). At the same total seed density, CMY showed preference for cowpea in C/A mixture (V=1.60). At a total seed density of 160 CMY showed preference for cowpea in C/G mixture (V=1.37) and in C/A mixture (V=7.23) and for greengram in G/A mixture (V=3.06).

DISCUSSION

The results of this study demonstrate that both strains of *Callosobruchus maculatus* showed a tendency to choose the host seeds in a frequency independent manner, indicating that they preferred a certain seed type for oviposition irrespective of its relative availability. However, slight change of preference of both these strains was observed in relation to the total seed density of the mixture. This change of preference can be explained in the context of the oviposition strategies the cowpea weevil adapt for maximising progeny fitness. The female of cowpea weevil secretes a chemical (oviposition marker) at the time of oviposition which makes egg-laden

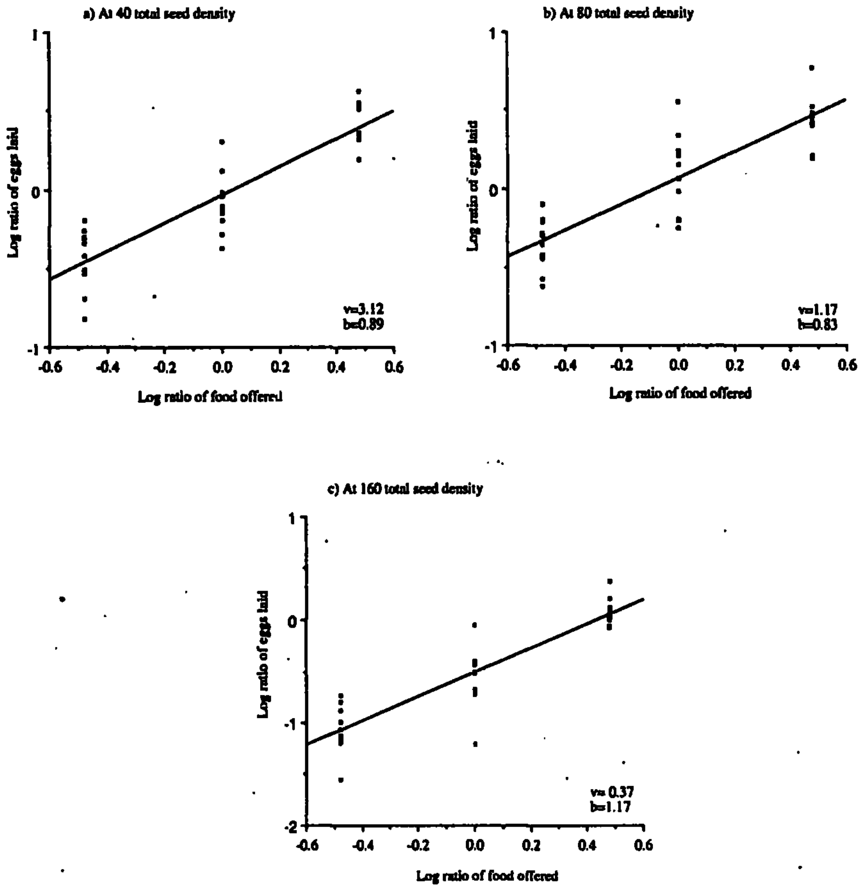

Fig. 2a: Log ratio of the number of eggs laid when females of *C. maculatus* Brazil strain were presented with seeds of cowpea and greengram in different ratios at: (a) 40, (b) 80, and (c) 160 total seed density. The line represents the regresion line for observed ratio of the eggs laid on two seed types.

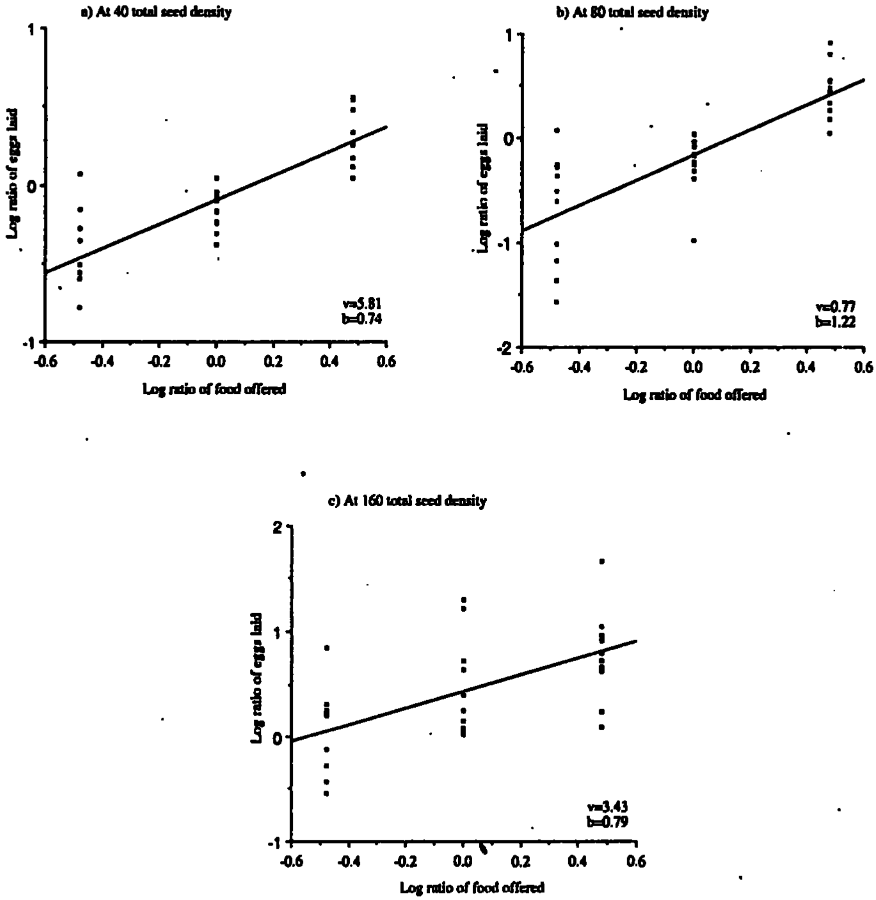

Fig. 2b: Log ratio of the number of eggs laid when females of *C. maculatus* Brazil strain were presented with seeds of cowpea and adzuki in different ratios at: (a) 40, (b) 80, and (c) 160 total seed density. The line represents the regresion line for observed ratio of the eggs laid on two seed types.

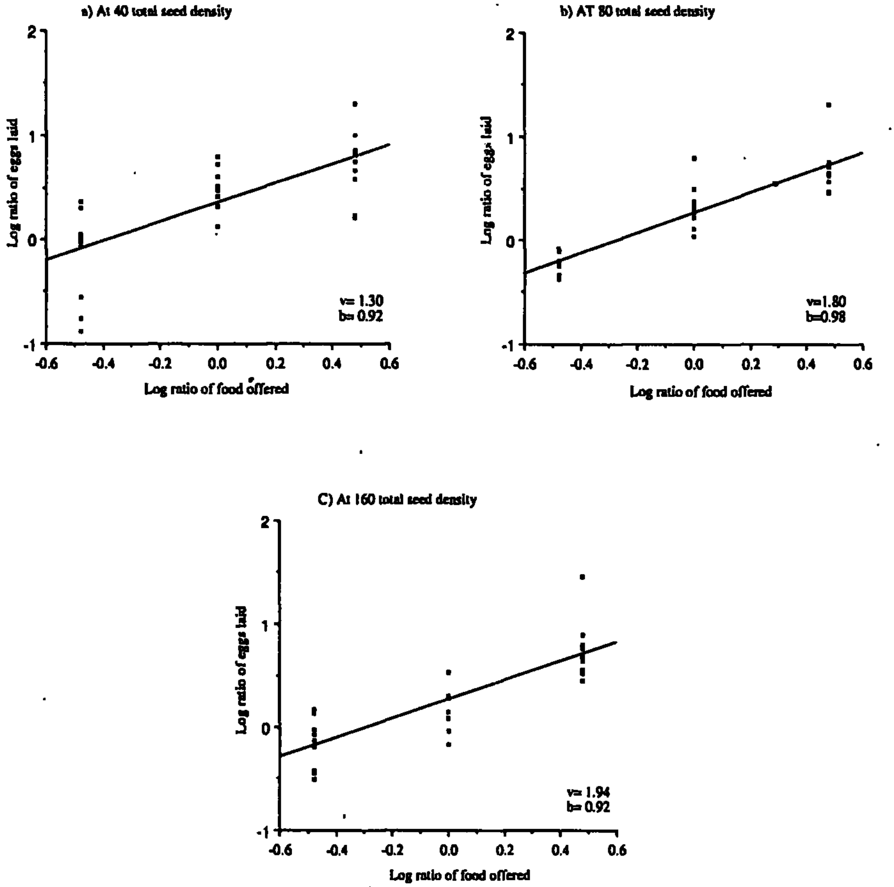

Fig. 2c: Log ratio of the number of eggs laid when females of *C. maculatus* Brazil strain were presented with seeds of greengram and adzuki in different ratios at: (a) 40, (b) 80, and (c) 160 total seed density. The line represents the regresion line for observed ratio of the eggs laid on two seed types.

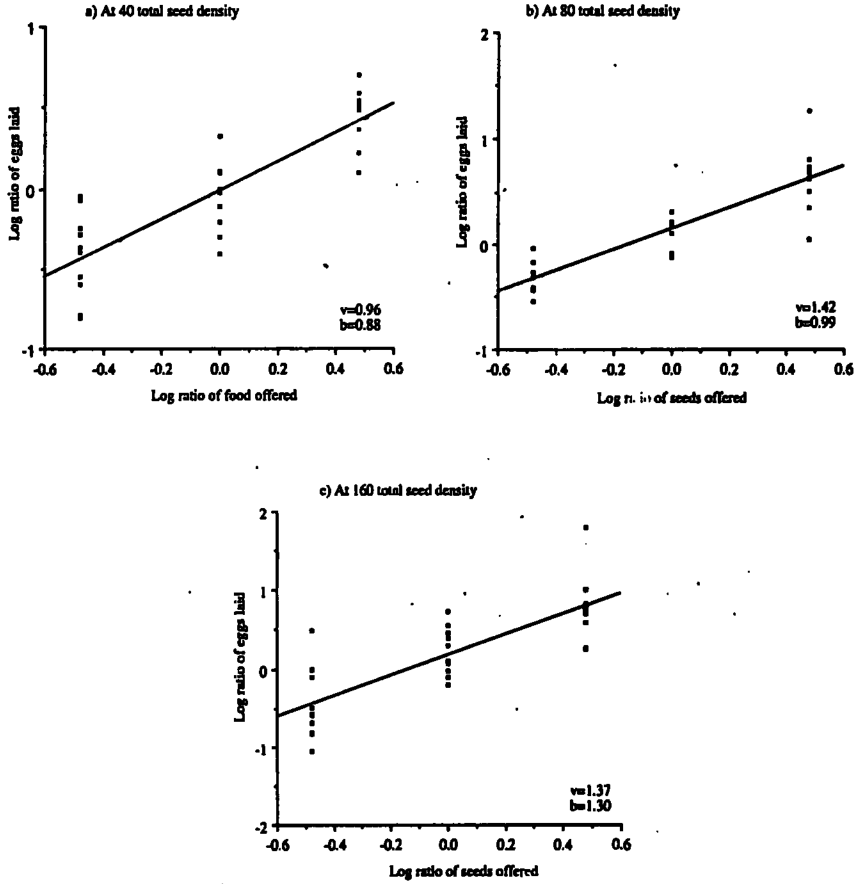

Fig. 3a: Log ratio of the number of eggs laid when females of *C. maculatus* Yemen strain were presented with seeds of cowpea and greengram in different ratios at: (a) 40, (b) 80, and (c) 160 total seed density. The line represents the regresion line for observed ratio of the eggs laid on two seed types.

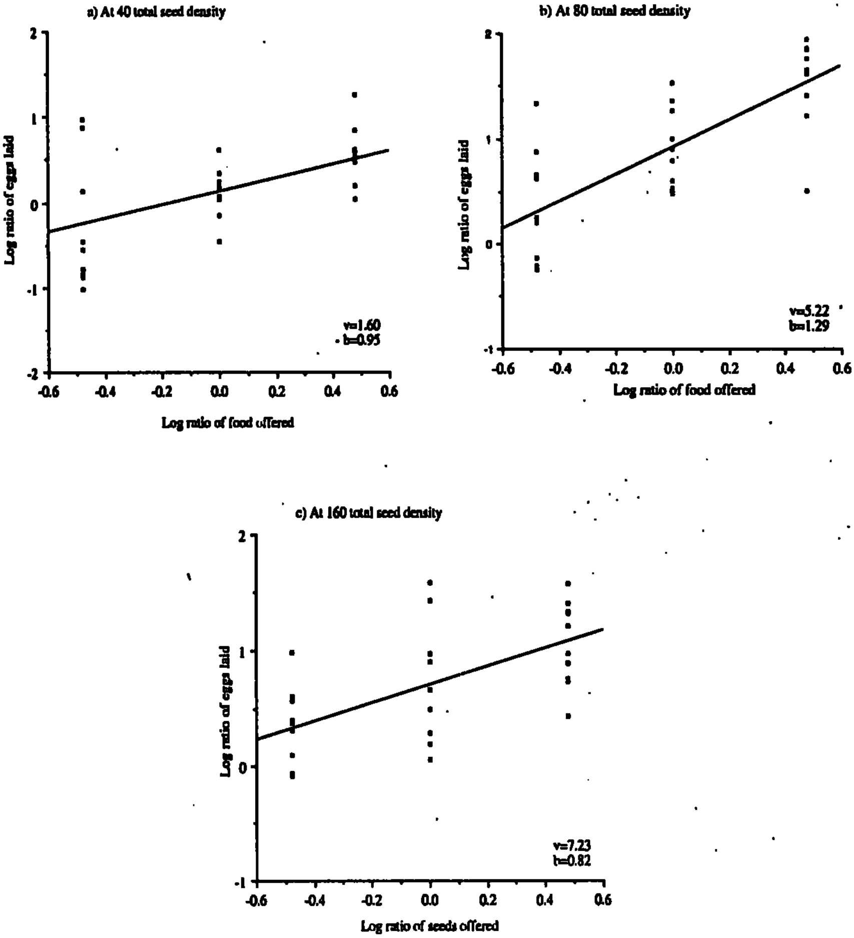

Fig. 3b: Log ratio of the number of eggs laid when females of *C. maculatus* Yemen strain were presented with seeds of cowpea and adzuki in different ratios at: (a) 40, (b) 80, and (c) 160 total seed density. The line represents the regresion line for observed ratio of the eggs laid on two seed types.

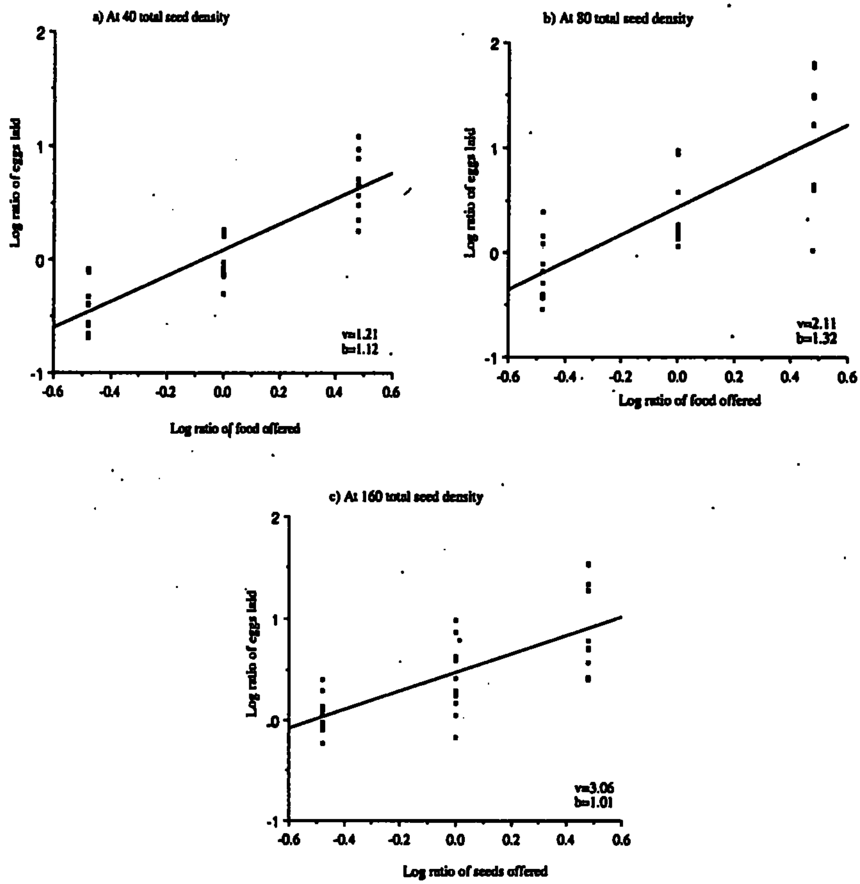

Fig. 3c: Log ratio of the number of eggs laid when females of *C. maculatus* Yemen strain were presented with seeds of greengram and adzuki in different ratios at: (a) 40, (b) 80, and (c) 160 total seed density. The line represents the regresion line for observed ratio of the eggs laid on two seed types.

seeds unattractive for further oviposition (Giga and Smith, 1985; Wijeratne, 1998). The cowpea weevil adapts this strategy to minimize the intraspecific competition among larvae and increase the progeny fitness (Smith and Lessells, 1985). Thus, at low seed densities the preference effects will be obscured because females will first oviposit on the preferred host seeds and when the relative attractiveness of the preferred host seeds declines due to oviposition marker, they move onto alternate host seeds for oviposition.

As the preference effects are obscured at low seed densities, the actual host seed preference should be evaluated at high seed densities. Accordingly at a seed density of 160, the ranking order of host seed preference of CMB is greengram, cowpea and adzuki, whereas that of CMY is cowpea, greengram and adzuki.

The larvae of cowpea weevil are unable to migrate between seeds and therefore placing of eggs by the female parent is all important in determining the survival prospects of the progeny. In this context female parent should choose the seeds to which the larvae are best adapted. In cowpea weevil, product of the body weight of the female at emergence and the number of females emerged from a single seed can be used as an index to measure the fitness value of the host seeds (Smith and Lessells 1985). It has been found out that cowpea seeds offer the most suitable substrate for larval development thereby increasing the progeny fitness (Wijeratne, 1991). Larval development in adzuki seed takes a longer period and the emerging adults have reduced body weight. Therefore, it can be stated that of the three types of seeds used in the experiment, adzuki was the most unsuitable seed in the context of progeny fitness. Thus the rational behind the frequency independent host selection of CMY is clear, as CMY preferred cowpea seeds which offers the highest progeny fitness. However, the host seed preference of CMB did not correspond to the progeny fitness, as CMB preferred greengram seed which does not offer as a good substrate as cowpea for progeny fitness. In a number of insects the relationship between the food preference and the progeny fitness has been found to be poor (Thompson, 1988; Via, 1990). This is because the behavioural pattern of the female that maximizes the fitness may be a consequence of something other than preference for the host which offers the most suitable substrate for progeny fitness.

In some insects so far studied including certain strains of *C. maculatus,* the host seed selection and the larval performance are genetically independent(Thompson and Pellmyr, 1991; Wasserman and Futuyama, 1981). This lack of correlation could be attributed to the fact that oviposition preference develop more rapidly than physiological adaptation or *vice versa.*

Frequency independent host selection maximizes the reward as inferior dietary items are completely dropped from the diet as the best food becomes available. Such a strategy is destabilising on the community structure and may play some role in the maintenance of feeding specializations (Partridge and Green, 1985). Frequency independent host seed selection of cowpea weevil suggest that the pattern of host utilization of this insect is less likely to undergo changes in food preference. However, an important point to be considered here is the oviposition marker of the weevil which makes occupied beans less attractive for further oviposition. If the scarcity of preferred host seeds continues there could be some behavioural adaptation to the previously less preferred host seeds. These findings also indicate the importance of total seed density in analysing the oviposition preference of cowpea weevil that responds to intraspecific competition.

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Table 1. Estimates of V (index of host seed preference) and b(index of frequency related host seed preference) with 95% confidence limits for *C. maculatus* Brazil strain. Different mixtures contain seeds on a fixed number basis.

Total seed density 40

Seed mixture	Overall preference		Frequency dependence	
	V	95% CI	b	95% CI
Cowpea-greengram	3.12	2.21-4.50	0.89	0.66-1.11
Cowpea-adzuki	5.81	4.06-6.61	0.74	0.41-1.07
Greengram-adzuki	1.30	1.13-1.50	0.92	0.76-1.07

Total seed density 80

Seed mixture	Overall preference		Frequency dependence	
	V	95% CI	b	95% CI
Cowpea-greengram	1.17	1.00-1.38	0.83	0.64-1.02
Cowpea-adzuki	0.77	0.61-0.97	1.22	0.88-1.56
Greengram-adzuki	1.80	1.56-2.08	0.98	0.98-1.18

Total seed density 160

Seed mixture	Overall preference		Frequency dependence	
	V	95% CI	b	95% CI
Cowpea-greengram	0.37	0.31-0.45	1.17	0.93-1.41
Cowpea-adzuki	3.43	2.47-4.78	0.79	0.37-1.21
Greengram-adzuki	1.94	1.60-2.34	0.92	0.67-1.17

Table 2. Estimates of V (index of host seed preference) and b(index of frequency related host seed preference) with 95% confidence limits for *C. maculatus* Yemen strain. Different mixtures contain seeds on a fixed number basis.

a. Total seed density 40

Seed mixture	Overall preference		Frequency dependence	
	V	95% CI	b	95% CI
Cowpea-greengram	0.96	0.80-1.16	0.88	0.66-1.11
Cowpea-adzuki	1.60	1.15-2.23	0.95	0.55-1.41
Greengram-adzuki	1.21	0.99-1.46	1.12	0.89-1.35

Total seed density 80

Seed mixture	Overall preference		Frequency dependence	
	V	95% CI	b	95% CI
Cowpea-greengram	1.42	1.17-1.71	0.99	0.88-1.09
Cowpea-adzuki	5.22	4.33-7.27	1.29	0.85-1.73
Greengram-adzuki	2.11	1.59-2.80	1.32	0.91-1.73

Total seed density 160

Seed mixture	Overall preference		Frequency dependence	
	V	95% CI	b	95% CI
Cowpea-greengram	1.37	1.02-1.83	1.30	0.91-1.69
Cowpea-adzuki	7.23	5.19-10.06	0.82	0.57-1.31
Greengram-adzuki	3.06	2.31-4.06	1.01	0.66-1.36