The 'neighbour effect' and its role in shaping the positional pattern of seed development in fruits: an illustration from the pods of *Erythrina suberosa*

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Plants exhibit certain intra-fruit positional patterns in the development of seeds. These patterns have been generally interpreted to be a consequence of resource and fertilization gradients. However, such positional patterns might also be shaped by the 'neighbour effect', wherein formation and development of a seed at any position might positively or negatively influence those of other seeds in the neighbourhood. In this article, we examine the role of such neighbour effect in shaping the positional pattern of seeds in the pods of *Erythrina suberosa*. The results suggest the existence of a positive neighbour effect leading to a higher frequency of seeds in contiguous positions.

INTRA-FRUIT abortion of seeds is a common feature in multiovulated species and has been attributed to various proximate factors such as resource[1-5] and pollen limitation[6-8], predation[9,10] and genetic load[11]. Seed abortion is also suggested to be an adaptive strategy to gain the dispersal advantage[12-14] or a consequence of overproduction of ovules as a bet-hedging strategy to overcome the unfavourable conditions[15]. Recently, seed abortion has been viewed as a consequence of evolutionary interactions such as sibling rivalry and parent–offspring conflict[14,16-20].

It is generally observed that plants in which ovules are linearly arranged within ovaries, exhibit intra-fruit positional bias in seed abortion. Seed abortion in these species occurs predominantly at the terminal and/or at the basal position[4,13,21] and occasionally randomly along the fruit[22]. Accordingly, several distinct positional patterns of seed development have been identified[21]. These patterns are generally argued to be a consequence of the interaction of resource and fertilization gradients operating within the fruit[21,23-25]. However, it is also likely that the seed development pattern is influenced by the 'neighbour effect', wherein develop-

ment of a seed at a given position would influence the probability of that in its neighbourhood. To our knowledge, this neighbour effect has not been critically addressed as an important factor contributing to the eventual patterns of seed development.

In this article, we analyse the contribution of such neighbour effect on the observed positional bias in seed development in the fruits of *Erythrina suberosa*. We show that the observed pattern of seed development in this species emerges due to a) preference of seeds to develop at certain positions, and b) a positive neighbour effect by which development of a seed at any given position favours that of another in the immediate neighbourhood.

The species and data

Erythrina suberosa, a deciduous legume tree, bears pods during spring in the dry- and moist-deciduous forests of peninsular India[26]. Predominantly two and occasionally one, three or four of the eight ovules develop to maturity such that number of seeds per pod exhibit a highly positively skewed distribution (Figure 1).

We collected fresh pods ($n = 52$) of *E. suberosa* from two closely growing adult trees in the moist-deciduous forest in BRT Wildlife Sanctuary, Mysore district. Two seeded pods, which dominated the sample ($n = 43$), were considered for further analysis. The positions of developing seeds within each pod were recorded starting from the base (pedicel end) to the tip (stigmatic end). Table 1 shows the positional frequency distribution of the 86 seeds. A strong positional preference is readily evident from the table; positions 3, 4 and 5 appear to be the most preferred for seed development.

The frequency distribution of seed-pair positions for the 43 pods is shown in Table 2. These seed-pair combinations are also categorized into neighbour and non-neighbour groups. Of the ten observed combinations, six are from neighbour accounting to 76.7% and
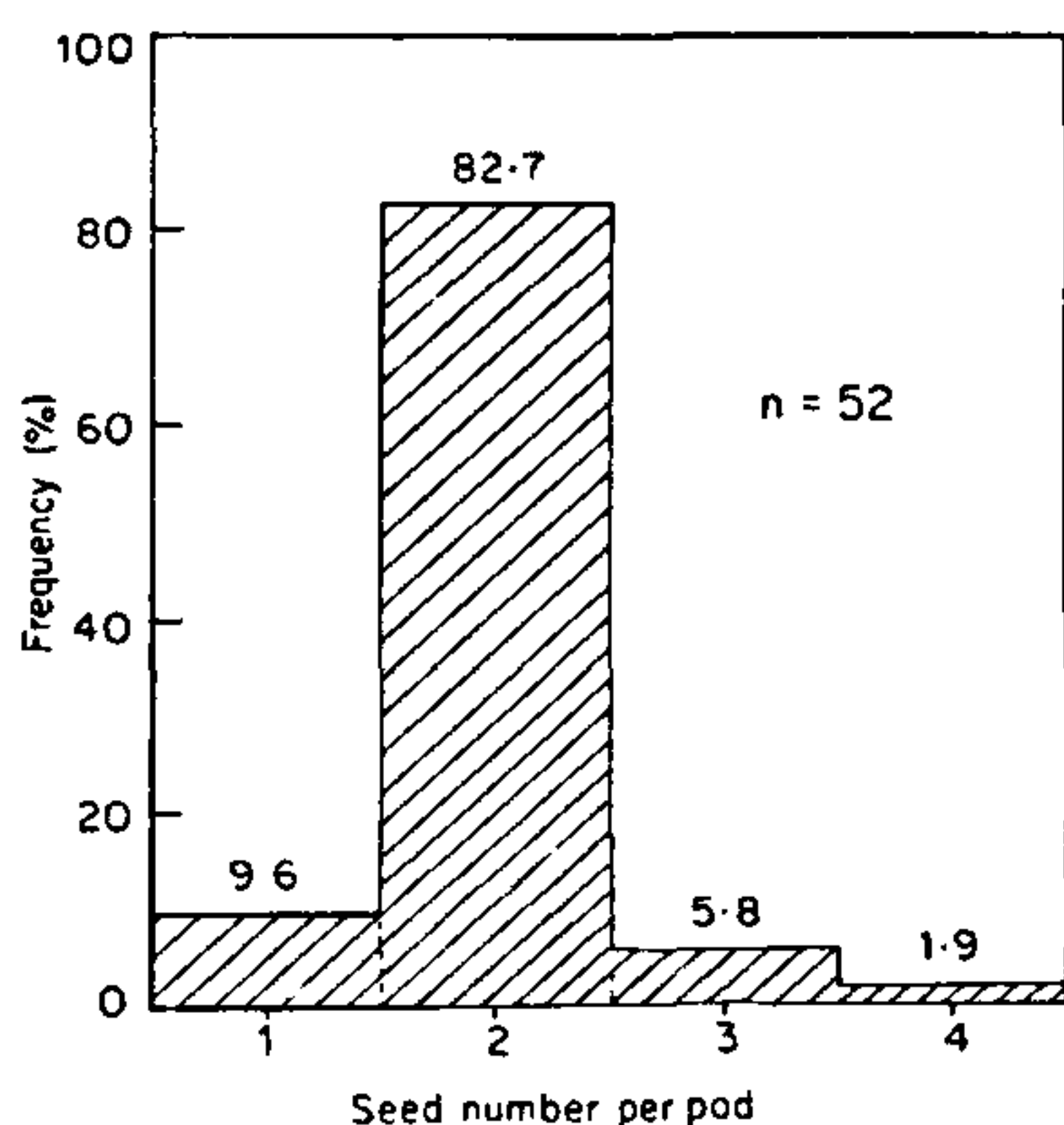
Figure 1. Frequency distribution of seeds per pod.

Table 1. Frequency distribution of seeds at the eight positions in the pod

Position from base	Frequency	Proportion (observed)	A priori preference (computed)
1	0	0	0.01
2	8	0.093	0.09
3	24	0.279	0.27
4	18	0.209	0.21
5	17	0 197	0.19
6	10	0 116	0.12
7	6	0.069	0.07
8	3	0.034	0.04

Table 2. The ten observed seed pairs and their frequency distributions

Seed-pair positions Seed #1	Seed #2	Neighbourhood attribute	Frequency	Proportion
2	3	Neighbour	6	0.140
2	5	Non-neighbour	2	0.047
3	4	Neighbour	10	0.233
3	5	Non-neighbour	3	0.070
3	6	Non-neighbour	4	0.093
3	7	Non-neighbour	1	0.023
4	5	Neighbour	8	0.186
5	6	Neighbour	4	0.093
6	7	Neighbour	2	0.047
7	8	Neighbour	3	0.070

the rest belong to non-neighbour category. In other words, there appears to be a strong preference for seeds to occur at the neighbouring positions. This prompted us to test if there exists any neighbour effect between the developing seeds in addition to positional preference. For this, we first computed the frequencies of all possible seed-pair combinations with and without any neighbour effect and compared them with those

observed. The model and the details of simulations are given below.

The model

Let p1, p2,...,p8 denote the probabilities of the first seed developing at positions 1, 2,...,8 respectively. These are the *a priori* probabilities and depending on the assumptions about the effect of the first seed (neighbour effect), the probability that the second seed develops at any of the remaining positions would vary. Thus, if there is no neighbour effect, the *relative* probabilities of the second seed developing in any of the remaining positions would remain the same. Alternatively, if the second seed develops only in the neighbouring positions of the first seed, then the probability at all the non-neighbour positions becomes, by definition, equal to zero. It is possible to work out the probability of occurrence of each possible arrangement of two seeds in the pod, if the *a priori* probabilities and the quantitative measure of the neighbour effect are known. These, in turn, would lead to the estimates of proportions of seeds which will be observed in positions $1, 2, \cdots, 8$. These in general would be different from the values of $p1, p2, \cdots, p8$.

For simplicity, consider a three-ovuled pod, where only two seeds develop. Let the *a priori* probability of the first seed to develop in positions 1, 2 and 3 be 1/4, 1/2 and 1/4 respectively. If there is no neighbour effect, the probability of obtaining pods with seeds at (1, 2) positions is given by $(1/4) \times (2/3)$, since the probability of the second seed developing at the 2nd and 3rd positions are now 2/3 and 1/3 respectively. The full computation shows that the probabilities of seeds being observed at positions 1, 2 and 3 are (7/24), (10/24) and 7/24) respectively, which are close to but clearly different from the *a priori* probabilities. Similar differences could arise if there is neighbour effect. These arguments and illustrations indicate that the *a priori* preferences have to be indirectly estimated using both the observed data and the underlying model; values of positional preferences directly computed from the data are unlikely to be exact. We describe below the procedure adopted for estimating the *a priori* probabilities and the parameters of different models of the neighbour effect.

Estimating a priori positional preferences: no neighbour effect

If the first seed is in position k, the second can be in any of the remaining seven positions. We assume that the *relative* probabilities at these positions remain unaffected. Thus, the probability of the second seed forming at any of the remaining positions (say i), given that the first

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seed is in position k, is

$$P(k,i) = pi/(p1 + p2 + \cdots + p8),$$

where the sum is over all positions except k. Hence,

$$P(k,i) = pi/(1 - pk) \tag{1}$$

Since there are eight possibilities for the first seed, seven for the second, and since from a pod the two cannot be distinguished, there are $8 \times 7/2 = 28$ observable categories of pods, based on the positions of the two seeds. Using equation (1), the expected frequencies in each of these categories can be calculated, and the chi-square value by comparing these with the observed frequencies (Table 2) obtained.

Though no seed was observed at position 1, we assume a low probability of 0.01 for p1, and the values of other probabilities were varied over the range from 0.01 to 1 in steps of 0.01 (keeping their sum equal to 1) and at each stage, the chi-square value for the comparison of observed versus expected frequencies calculated. The set of p values which minimizes this quantity was taken to be the best estimate of the *a priori* probabilities. The number of combinations to be searched could be substantially reduced by imposing an approximate constraint of a single mode, i.e. by imposing the restrictions p1 < p2, p2 < p3, p8 < p7 and p7 < p6.

Neighbour effect: linear decrease with increasing separation of seeds

If the first seed has neighbour effect on the development of the second seed, and if the former is at position k, then the probability of the latter developing at position i will be a function of both a) the *a priori* probability pi, and b) the neighbour effect of the seed at position k on that at i. We model the latter, the neighbour effect, by the parameter Wi, which is the weight attached to the probability pi. The probability for the second seed to be in position i is given by

$$P(k,i) = Wi \cdot pi/(W1 \cdot p1 + W2 \cdot p2 + \cdots + W8 \cdot p8),$$

where the sum is carried out over all positions except k.

If the first seed is in position k, and when its influence is linear, the weightage for the probability of the second seed to develop in position i is given by

$$Wi = 1 - C \cdot x,$$

where x is the separation given by

$$x = ABS(k - i) - 1.$$

If Wi attained a negative value, it was taken to be equal to zero. Here, x ranges from 0 (nearest neighbour) to 6 (seeds in positions 1 and 8). The parameter C in the model indicates the rate at which the weightage decreases with distance.

Nonlinear decrease with increasing separation of seeds

Two nonlinear forms of dependence of weightages on separation were attempted. The exponential model.

$$Wi = \exp(-C \cdot x)$$

and the power-law model

$$Wi = (1 - x/7)^C.$$

The definition of x, and the procedure for computing probabilities of occurrence for various positions for the pairs of seeds are the same as described above.

Simulations

During the computations of chi-square values, many of the expected frequencies were seen to be smaller than 5, suggesting that inferences based on the chi-square distribution may not be strictly valid. To circumvent this difficulty, the distribution of seeds was simulated for each of the four models. Thus, 1000 sets of 43 pods were simulated. For each simulation, the frequency of occurrence for each of the 28 classes was obtained. A chi-square value comparing this frequency with that expected based on the model was computed. The simulation-based distribution of chi-squared values, thus obtained, was used to test the statistical significance of the chi-squared values obtained by comparing models with the data.

Results

(i) A model assuming a constant preference at all the positions is clearly ruled out (chi-square $= 125$, $df = 28$, $p < 0.01$).

(ii) In the model assuming positional preferences, but no neighbour effect, the probabilities p1, p2,...,p8 have to be estimated from the data itself. Since these have to add to 1, there is a decrease of only 7 degrees of freedom, leaving 21 df. Using equation (1), the expected frequencies in each of the 28 classes were computed and compared with the observed ones given in Table 2. The chi-square statistic of goodness of fit was 53, ($p < 0.01$), indicating the fit to be very unsatisfactory. The

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result thus clearly indicates the existence of the neighbour effect.

(iii) For the linear model, the minimum value of chi-square was 23.5 at the value of $C = 0.30$ and the fit is not unsatisfactory ($df = 20$, $p < 0.90$). Still lower values of chi-square could be obtained for $C = 0.50$ and above. However, these values of C led to expected frequencies being zero for pairs which were actually observed in the data. Hence, these were discarded, and $C = 0.30$, which shows a local minimum, was retained.

(iv) Amongst the nonlinear models, the exponential model led to a minimum chi-square value of 21.4, at $C = 0.70$ ($df = 20$, $p < 0.70$). For the power model, the minimum value of chi-square was 21.7 at $C = 3.9$ ($df = 20$, $p < 0.70$). Both these models thus give a reasonable fit to the data. However, it is not possible to distinguish between the linear and the two non-linear models based on their performance, as the differences between the corresponding chi-square values are small. As seen from Figure 2, the weightages in all the three models show a sharp decrease with increasing separation between the seed positions.

(v) Results from simulation are consistent with those obtained from a comparison of chi-square values. For the 'no neighbour effect' model, the simulated chi-square exceeded the observed in only 25 of the 1000 simulations, confirming a poor fit. For the linear, exponential and power models, the chi-square value exceeded in 283, 500 and 455 simulations respectively, indicating a reasonable degree of fit.

Discussion

An analysis of the patterns of seed positions in the two seeded pods of *E. suberosa* indicates a distinct preference for seeds to develop at positions 3, 4 and 5 compared to the remaining five positions.

Our results also illustrate that the observed positional preference of seeds is further modulated by the neighbour effect. The neighbour effect can be expected to have direct consequences to fitness components such as the number and weight of seeds, dispersal efficiency of fruits and the eventual seedling fitness. It would be interesting to understand the mechanism through which such neighbour effect operates. The development of the first seed is likely to trigger a higher resource flow towards its position in the pod. Consequently, fertilized ovules in its neighbourhood are more likely to be incidental beneficiaries of such increased flow, facilitating their development. Such a process might lead to the establishment of weaker genotypes by the increased sink facilitation created by the genetically dominant
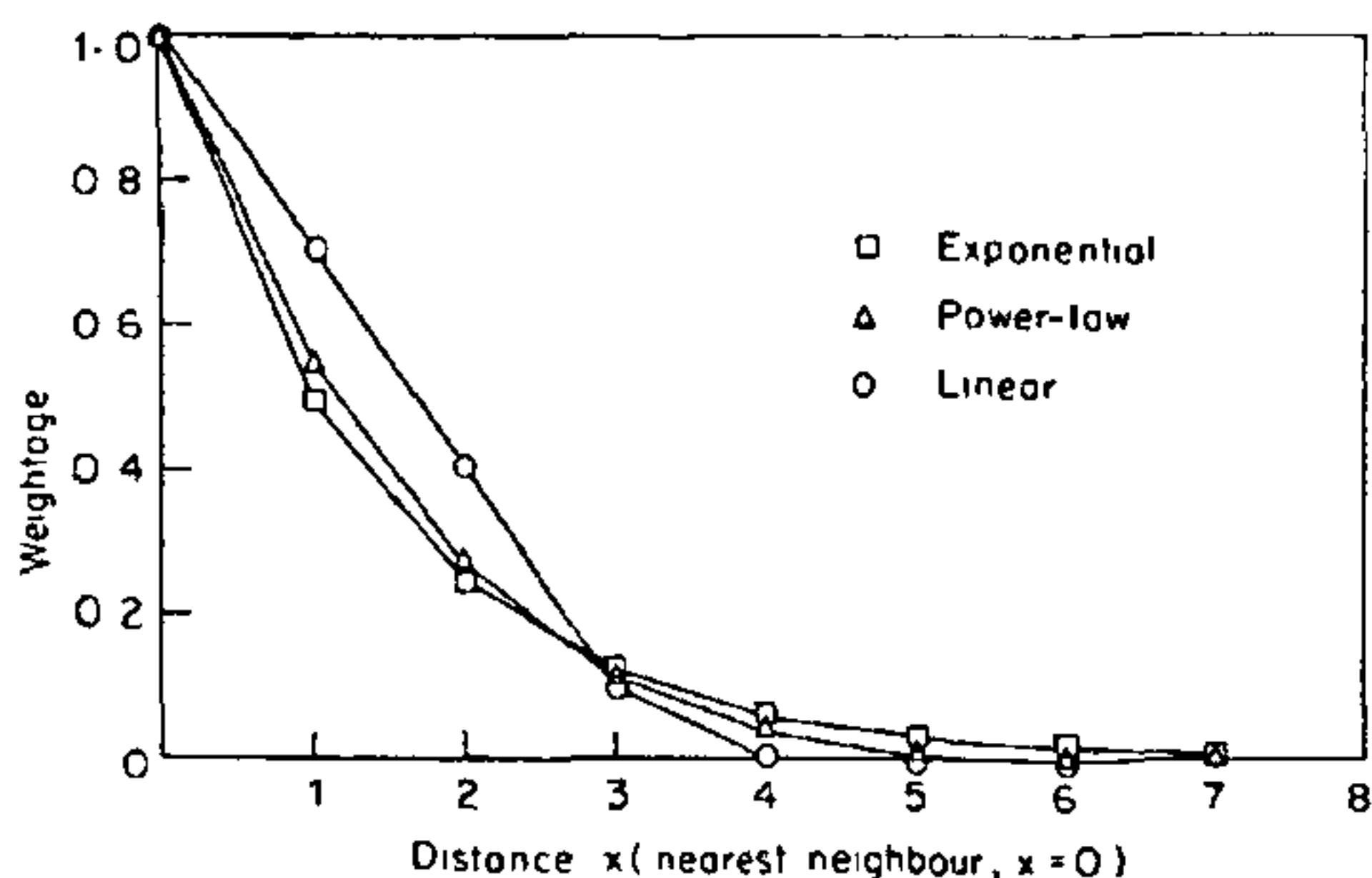
Figure 2. Reduction of weightage with distance in the three models.

seed in the neighbourhood. However, if neighbouring ovules happen to be fertilized by genetically superior pollen grains, the resulting seeds may together establish a domain of strong sink drawing ability. In other words, such seeds mutually favour their development over others, resulting in the neighbour effect. In the light of these multiplicity of interactions, which are likely to have a strong influence on the fitness, it seems worthwhile to understand the evolutionary significance of the neighbour effect.

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Influence of a biotic stress (leaf curl viral infection) on the sex ratio and resource allocation in *Momordica tuberosa* (Roxb.) Cogn. — A monoecious perennial herb

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We have examined the effect of a biotic stress, leaf curl viral infection, on the floral sex ratio and resource allocation patterns in a monoecious herb, *Momordica tuberosa*. Diseased plants had significantly less number of female flowers and consequently exhibited a predominant male biased floral sex ratio. Nearly 26 per cent of the diseased plants were completely males. Resource allocation pattern to the vegetative and reproductive units remained unaltered in diseased plants, however, a greater proportion of reproductive resources was allocated to male function. We discuss the adaptive significance of such shift in the floral sex ratio and resource allocation between the sexes.

MONOECIOUS plants alter their sex ratio (number of male to female flowers) and allocation of resource to reproductive structures as an adaptive response to their resource status[1-4], habit[5], alterations in environmental conditions such as soil moisture[6], light[6] and to altitude[7]. Such adaptive sex ratio changes in response to abiotic stresses have also been confirmed under carefully controlled conditions of light and soil moisture regimes[6].

Very little is known about the sex ratio variations in response to biotic stresses such as pest and disease incidence. Bazzaz[8] reported a male biased sex ratio of tall *Ambrosia trifida* plants compared to short as a response to a higher degree of seed predation by insects. Infection of a fungal pathogen, *Ustilago spp.* has long been known to reverse the sex in a dioecious *Lychenis* and monoecious *Zea mays*[9]. To the extent that biotic stresses also affect the resource status of the plant, it may be expected that the response of plants and their resource allocation patterns between sexes is similar to that observed under abiotic stresses. In this article, we report the effect of biotic stress induced by a viral infection (leaf curl) on the sex ratio and resource allocation to vegetative and reproductive structures in *Momordica tuberosa* (Roxb.) Cogn., in its natural habitat.

System

Momordica tuberosa (Roxb.) Cogn. (Syn: *Luffa tuberosa*) (Cucurbitaceae) is a monoecious, insect-pollinated, prostrate, tuber forming perennial herb. It colonizes the fallow and cultivated lands of arid regions of southern India. Many scandant branches radiate from a common tuber. Both primary and secondary branches bear at each node either male (in 2–5 flowered raceme) or solitary female flowers. The light yellowish male flowers are larger ($n=74$, $\bar{X}=2.05\pm0.03$ cm) compared to females ($n=64$; $\bar{X}=1.41\pm0.02$ cm; $P<0.01$) in diameter and possess a dark yellow spot at the base of the petals. Female flowers produce four to five ovules arranged linearly. About 2 to 3 per cent of the plants, including seedlings, are generally infected by leaf curl virus and transmitted through white flies (*Bemisia tabacii*) (personal observation). The infected plants are stunted with pale yellowish crinkled leaves and are thus readily distinguishable from healthy plants.

The study was conducted at the Regional Research Station, University campus, Raichur (16° 15′N, 77° 20′E; 389 m above MSL), Karnataka, India.