

Dear Sir,

I have gone through "Evolution of Polyembryony: Consequences to the Fitness of the Mother and the Offspring" by K.N.Ganeshiah et al. The contention of the authors is that polyembryony - the production, in parallel with the fertilised egg, of additional embryos derived entirely from maternal tissue - is a strategy favoured by the maternal genome to counter any tendency on the part of the zygote to limit the number of its siblings (especially when there is multiple pollination). This contention, supported by quantitative models, is both plausible and interesting, as are the observations presented in Tables 1a, 1b and 3a. I wish I could leave it at that. Unfortunately, the paper is unreadable as it stands, and needs to be re-worked drastically if it is to be considered for publication. As I have made extensive comments in the text, I will just summarise my main criticisms here:

1. The mathematics is opaque rather than transparent. At least the genetic analysis should be cast in a form which readers other than quantitative geneticists can appreciate (if not understand). Also, it will help a great deal if in each case the result of mathematical calculation is re-stated in words.
2. The text is repetitive, the language turgid, and the notation baffling when not confusing.

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3. (p.11, equation 6) Why should the optimal investment of maternal resources in the zygote be the same whether or not a "polyembryo" is formed? One senses here the implicit assumption that the mother allocates resources "as if" for the zygote alone, with some of these resources subsequently being "hijacked" by the polyembryo. Is this what the authors have in mind? If yes, how do they square such "hijacking" with the conclusion that polyembryony is likely to evolve via selection acting on the maternal genome? If not, what do they really have in mind?

4. The statement on p.23-24 (asserting a negative correlation between polyembryos per seed and number of seeds per fruit) should be supported by a table.

5. The authors should take a look at Haig & Westoby, Am. Natur. 134 p.147, 1989.

I am returning my copy of the MS.

B. Response to the referee's comments on the manuscript

(Numbers refer to corresponding ones in the manuscript)

1. This paragraph sets out an important point- that PE is different from apomixis and adventitious embryony and hence evolution of polyembryony cannot be considered tantamount to the evolution of asexual reproduction via apomictic or adventitive reproduction. We have therefore retained this paragraph.
2. Yes, as far as the genotype of the embryo is considered.
3. As treated in the paper, the additional embryo is regarded as competing for the resource allocated to the zygotic offspring. Thus, we do not visualize a situation where the additional embryo provides support to the zygote. Hence, formation of additional embryo from maternal tissues of the embryo sac contributes to the fitness of the mother than to the sexually produced zygote.
4. The point made is reasonable in light of our remarks above (#3)
5. Our concern in the paper is about the evolution of polyembryos from maternal tissues (mainly, nucellar) and its consequences to the fitness of maternal parent and offspring.
6. The power of the fitness function as evident from a number of empirical studies (for instance, see the references cited in p382 of McBinley et. al.(1987), Am.Nat. 130:370-398; a xerox copy of the page is enclosed alongwith ) is less than 1. This is true for any parameter of fitness measured, example, leaf area of seedlings, seedling vigour, plant height etc. Hence the limit  $x < 1$ .
7. Proof is now included in the text (see p6-p7).
8. Incorporated.
9. The nucellus is a maternal tissue component of the embryo sac. Nucellar embryos are formed from the differentiation and subsequent development of this tissue; this in fact has been mentioned in the introduction. Thus to the extent that the maternal sporophyte tissue

is heterozygous, we expect the same level of heterozygosity to be exhibited by the nucellar embryo.

10. Incorporated.

11. The choice of the subscripts in the notations,  $r_{zm}$  and  $r_{zz}$  is deliberate: z refers to zygote and m to the maternal parent.

12. Incorporated.

13. Bt and Ct have now been changed to B and C.

14. See reply to referee comment 3 of the comment sheet.

Secondly, we have considered a more general expression, involving unequal investment in the nucellar and zygotic embryos; the referee discusses a special case when these two are equal.

Thirdly, the values of  $x$  and  $c$  can be such that (e.g.  $x=0.75$  and  $c=0.25$ ) we get  $N^*=1$  but  $M^* < 1$ . The referee's point that PE is always (globally) preferred is thus invalid.

15. Corrected.

16. Incorporated.

17. Citation is correct.

18. Regret misunderstanding caused. Text is rephrased.

19. Hamilton derives the formula from first principles in the specific context of using it for computing inclusive fitness, hence it is appropriate to cite his 1972 review.

20. Clutch is used in plant literature (e.g., Levin, 1986; Uma Shaanker et. al., 1990)

21. The sentence is deleted.

22. Incorporated.

23. By the definition of the word infinite, no real species has an infinite number of ovules. For that matter, no species has an infinite population either. The term 'infinite' is normally used to indicate a very large number, a convention which should be normally known to anyone who has even a passing knowledge of application of mathematics to other branches of science.

Secondly, as seen from eqn 8, the discussion just after eqn. 8 and from fig.2 ,  $B_t/C_t$  CANNOT exceed 1. Equation 9 points out the condition necessary for PE to evolve; it does NOT assert that the condition IS satisfied. The referee's contention that  $H=1$  is sufficient is rather absurd.

24. Corrected.

25. We have deleted this paragraph.

26. We mean that maternal parent favours the evolution of PE while the zygote (sexual offspring ) does not.

27. As explained earlier, a different notation (U, V and W), which cannot be easily confused with B and C, has now been adopted.

28. Suitably modified.

29. Expression 15 derives a condition. It does NOT assert that the condition is satisfied. In fact, in the framework of the present model, the condition cannot be satisfied. This has been clearly explained at the end of the paragraph. Thus there is no discrepancy in the first place which has to be reconciled.

30. Data now provided; see Figure 10 as well as the accompanying text.

31. The simple one-locus-two-allele models of diploid populations can be described by two variables, say the frequencies of any of the two (of the three possible) genotypes. Often, when Hardy-Wienberg law holds, only one variable, say the gene frequency of one of the alleles, is adequate for describing the dynamics. If one of the alleles is rare (say with frequency  $p$  close to zero), then the frequency of the heterozygote involves terms to first order in  $p$ , and that of the homozygote of the rare allele, terms to second order in  $p$ . In such a case, the mathematics is relatively simple, and one can use either the gene frequency  $p$ , or the heterozygote frequency  $p/2$  to describe the dynamics of gene frequency change. This, for example, is done in B1c.

On the other hand, in certain other cases (generally involving inbreeding), the frequency of the homozygote of the rare allele also involves terms to first order in  $p$ . The dynamics of genotypic frequencies thus becomes a problem involving two variables. This is exactly what happens in many of the situations described in the appendix, and the simplification suggested by the authors is not valid. In other words, in simple models, the frequency of the homozygotes of the rare allele, even if present to first order, is invariably reduced to the second order in the next generation. In more complex models such as dealt with here, they continue to be present to first order in the next generation. In the interest of rigour, we feel compelled to retain the linearized stability analysis involving eigenanalysis of  $2 \times 2$  matrices in the m/s.

Reply to referee's comments (referee 1)

We have carefully gone through the remarks of the referee. We were unable to find any specific comment about how the m/s can be altered. The referee seems to hold a somewhat different opinion about the utility of the kind of mathematical models presented in the m/s than us, and an objective assessment of the two points of view does not seem to be possible.

We have gone through Lovett Doust and Lovett Doust's 1988 paper. On p19, they remark that virtually nothing is clear about the function and role of polyembryony, much less its evolutionary consequence. We have accordingly cited this reference in support of our view that the evolution of polyembryony is poorly understood.

It is true that in testing the predictions of our models, we have lumped species with different characteristics together; the point is that despite the 'noise' which is introduced due to such a course of action, the 'signal' is clearly discernible, as shown by the statistical test of significance.

In summary, the referee's comments are neither specific nor convincing enough for us to make any changes in the m/s.

## Reply to referee's comments (referee 2)

We thank the referee for going through the m/s very carefully, and making many suggestions towards improving the presentation. We have incorporated most of the suggestions, and responded to the comments. In the instances where we do not agree with the referee's suggestions, we have explained our point of view in detail.

### A. Response to referee's covering letter.

(Numbers refer to corresponding numbers in the referee's comment sheet)

#### 1. Comments about the opacity of the mathematics used in the m/s

Additional details about the computations involving changes in the genotypic frequencies from one generation to the next have been included in the appendix (B1a); a similar approach is valid for rest of the calculations.

#### 2. We have now deleted certain redundant statements/sentences and have tried to be more explicit in the places indicated.

We agree with some of the remarks made by the referee regarding the notation employed in the m/s ; some of it is indeed awkward. We have altered some of the notation. In other places, we are unable to agree to the referee's suggestion. For example, in the appendix B, where we use  $n, y, z$ , the referee suggests  $x, y, z$ . However, (a)  $n$  seems more apt to describe the non-polyembryonic genotype NN and (b) we have already used  $x$  to denote the power of the fitness function, and we would like to be consistent with the earlier literature.

As to benefit and costs, we have now used B and C, instead of Bt and Ct used earlier. For the fitnesses, instead of the earlier A, B and C, we have now used U, V and W. While not intuitively obvious as to what quantity these symbols stand for, this choice at least removes the possible confusion with the notation for costs and benefits.

As for the rest of our notation, according to us, the clarity is adequate. We would like to use a single character symbol for the quantities which appear in the computation of genotypic frequencies, and we would like to be consistent with the notation used in the earlier literature. Within these constraints, we would be willing to consider any alternative notation if the referee specifically recommends it in the interest of clarity.

3. We have indeed started from the assumption that given "n" seeds per pod, the mother optimizes her allocation of resources to each seed (implying that there are no extra resources left with the maternal parent). ~~This~~, <sup>in fact, in</sup> ~~the~~ the event of an additional embryo (nucellar embryo) being formed in the seed, the already optimized resource <sup>(See assumption 2)</sup> is re-allocated between the zygote (sexual offspring) and the additional nucellar embryo. <sup>Further, the</sup> ~~The~~ optimal allocation by the mother to the entire fruit would remain valid even after the formation of the additional embryo considering certain important parameters of survival such as the dispersal efficiency of the fruit.)

go to p8

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5. Haig and Westoby (1989) is now cited in the text.

Reply to referee's comments (referee 1)

We have carefully gone through the remarks of the referee. We were unable to find any specific comment about how the m/s can be altered. The referee seems to hold a somewhat different opinion about the utility of the kind of mathematical models presented in the m/s than us, and an objective assessment of the two points of view does not seem to be possible.

We ~~have been~~<sup>are</sup> aware of the work of the authors whose names have been cited. In our opinion, we see no need to alter the list of references cited by us.

It is true that in testing the predictions of our models, we have lumped species with different characteristics together; the point is that despite the 'noise' which is introduced due to such a course of action, the 'signal' is clearly discernible, as shown by the statistical test of significance.

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7. Proof is now included in the text. (see p 7)

(see enclosed sheet)

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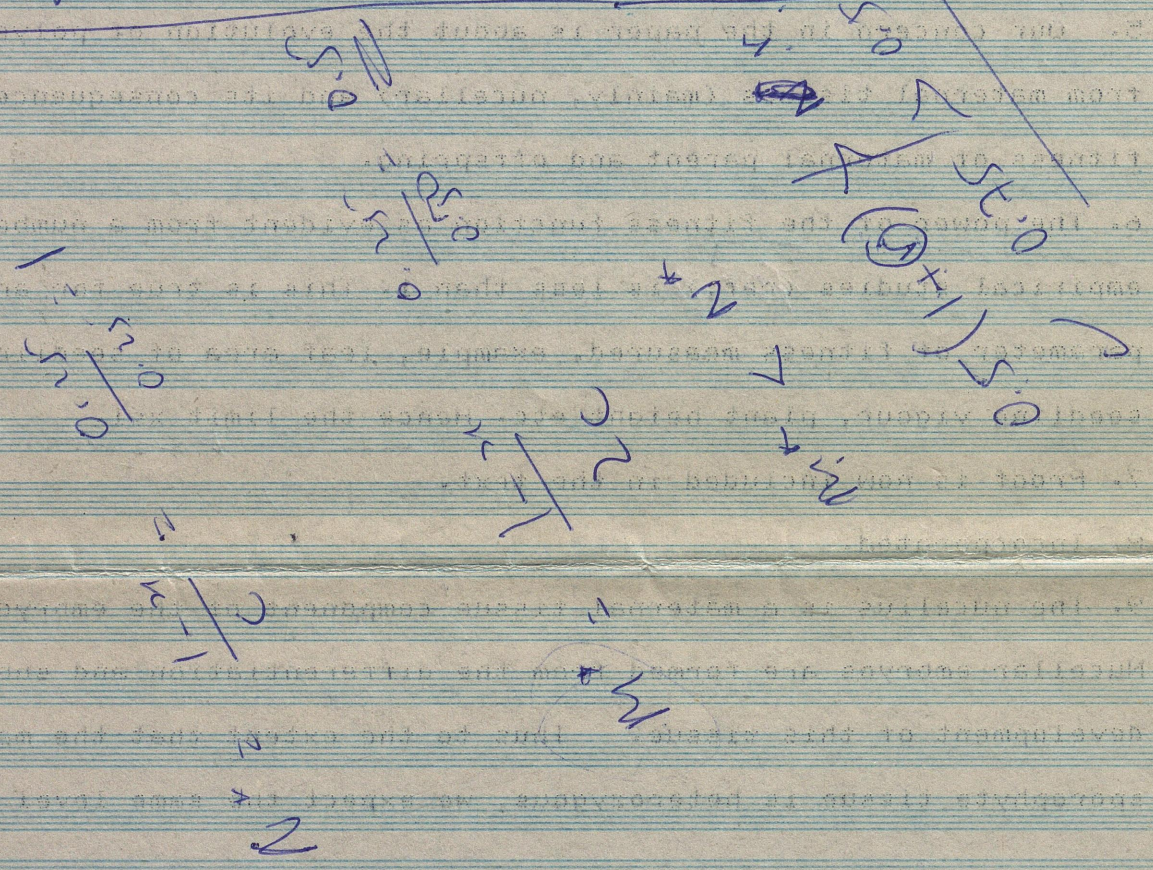
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$$M^*(1+r) > N^*r \quad \text{will be satisfied}$$

ii Polyeubryos should always be selected. I suggest that you kindly check this point



Dear Joshi, If ~~will~~ call ~~you~~ I don't reach you by phone or person by 12.00 noon assume that this point stands.

RHS

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complex models such as dealt with here, they continue to be present to first order in the next generation. In the interest of rigour, we feel compelled to retain the linearized stability analysis involving eigenanalysis of  $2 \times 2$  matrices in the m/s.