

## SHORT COMMUNICATION

**Evidence for epistasis: reply to Trouve *et al.***

D. EBERT,\* P. SALATHÉ† &amp; T. J. KAWECKI\*

\*Département de biologie, Ecologie et évolution, Université de Fribourg, Fribourg, Switzerland

†2-600 Queen Street, Newmarket, Ontario L3Y 2H9, Canada

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crosses**Abstract**

Salathé and Ebert (2003, *J. Evol. Biol.* **16**: 976–985) have shown that the mean logarithmic fitness of *Daphnia magna* clones declined faster than linearly with increasing inbreeding coefficient  $F$ . They interpreted this result as evidence for synergistic epistasis. Trouve *et al.* (2004, *J. Evol. Biol.*, doi: 10.1111/j.1420-9101.2004.00755.x) suggested that hybrid vigour could be an alternative explanation for this finding. We use a population genetic model to support the original claim that the marked deviation from linearity cannot be explained without epistasis. We further argue that the relevant reference population is the metapopulation and not the subpopulation. Taken together, we believe that synergistic epistasis between recessive deleterious alleles segregating in the *D. magna* metapopulation is the most likely explanation for the finding of Salathé and Ebert.

In a recent paper, Salathé & Ebert (2003) have shown that the mean logarithmic fitness of *Daphnia* clones originating from a particular crossing scheme (Fig. 1 in Salathé & Ebert, 2003) declined faster than linearly with increasing inbreeding coefficient  $F$ . They interpreted this result as evidence for synergistic epistasis between deleterious alleles at different loci; they asserted that in the absence of epistasis the relationship between  $F$  and mean log fitness should be linear. Trouve *et al.* (2004) notice that the three crosses whose fitness was assayed not only differed with respect to  $F$ , but also with respect to the expected contribution of the two parental lines to their genomes. They suggest that this should have led to hybrid vigour, which would have benefited certain types of crosses in our breeding design more than others. They claim that this hybrid vigour could be an alternative explanation for the nonlinearity of the relationship between mean log fitness and  $F$ . Hence, according to Trouve *et al.*, the conclusion about synergistic epistasis is not fully supported. Because hybrid vigour has been demonstrated in the *Daphnia magna* metapopulation from which Salathé & Ebert (2003) took their material (Ebert *et al.*, 2002), their idea needs careful consideration. The comments by Trouve *et al.* do not question our

second main conclusion, namely that there is no synergism between inbreeding and parasitism.

First we would like to point out that epistasis (like dominance) describes relationships between fitnesses (or phenotypes) of different genotypes. In contrast, hybrid vigour (like inbreeding depression) refers to a comparison of mean fitness between populations that differ in genotype frequencies. Both dominance and epistasis can contribute to both hybrid vigour and inbreeding depression (Lynch & Walsh, 1998). The magnitude of hybrid vigour and inbreeding depression depend on the choice of the reference population (see below for our choice of reference population). However, disregarding the reference population, whether the differences in fitness between lines assayed by Salathé *et al.* are described as inbreeding depression or hybrid vigour is of secondary importance for the conclusion about epistasis. The crucial question is whether the pattern in Fig. 4 of Salathé & Ebert (2003) can be explained without invoking epistasis.

**Can the results be explained assuming no epistasis?**

As pointed by Trouve *et al.*, the lines assayed by Salathé and Ebert differ in the genetic background: all genes of line  $G_{2s}$  descend from parental line  $P_s$  whereas in the backcrossed lines  $G_{2x}$  and  $G_{3x}$  a part of one haploid set of genes (respectively 50 and 25%) come from parental line  $P_x$ . A legitimate concern, not addressed in Salathé & Ebert

*Correspondence:* Dieter Ebert, Département de biologie, Ecologie et évolution, Université de Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland.

Tel.: +41 (0)26 300 88 69; fax: +41 (0)26 300 96 98;  
e-mail: dieter.ebert@unifr.ch

(2003), is whether the linear relationship between  $F$  and fitness expected under no epistasis still holds in this case.

We will show that, although it does not hold in general, this relationship does hold for the three specific lines assayed by Salathé and Ebert. Specifically, we will show that without epistasis (i.e. with log fitness additive across loci),  $\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}} = \bar{w}_{G_{3x}} - \bar{w}_{G_{2s}}$  holds for an arbitrary set of genotypic fitness values, where  $w_i$  is expected log fitness of cross  $i$  as defined by pedigree in Fig. 1 in Salathé & Ebert (2003). Because the absence of epistasis means that effects on log fitness are additive across loci, it suffices to show that this relationship holds for each locus separately. Consider thus a single locus and, without loss of generality, denote the two alleles carried by line  $P_s$   $A^1$  and  $A^2$ , and the allele passed on by line  $P_x$  to line  $G_{1x}$   $A^3$ . Of course,  $A^1$  and  $A^2$  will be identical if  $P_s$  is a homozygote, and  $A^3$  may also be identical to one or both alleles carried by  $P_s$ . Let  $q_i^{kl}$  be the probability that line  $i$  carries genotype  $A^k A^l$ , and let  $\mathbf{q}_i = [q_i^{11}, q_i^{12}, q_i^{22}, q_i^{13}, q_i^{23}]$  be the vector of these probabilities for all genotypes possible for a given descendant line. Note that, by symmetry and definition,  $q_i^{11} = q_i^{22} = F_i/2$ , where  $F_i$  is the inbreeding coefficient of line  $i$  relative to  $P_s$ . For the doubly selfed offspring  $G_{2s}$  it can be easily seen from the pedigree in Fig. 1 in Salathé & Ebert (2003) that

$$\mathbf{q}_{G_{2s}} = \left[ \frac{3}{8}, \frac{1}{4}, \frac{3}{8}, 0, 0 \right]. \quad (1)$$

The backcross  $G_{2x}$  has a 50% probability of receiving  $A^3$ , in which case the other allele is equally likely to be  $A^1$  or  $A^2$ . In the remaining 50% of cases, line  $G_{2x}$  will have received both alleles from parental line  $P_s$ ; they will be identical by descent in half of those cases, so

$$\mathbf{q}_{G_{2x}} = \left[ \frac{1}{8}, \frac{1}{4}, \frac{1}{8}, \frac{1}{4}, \frac{1}{4} \right], \quad (2)$$

consistent with  $F_{G_{2x}} = 0.25$ . The second backcross  $G_{3x}$  has only a 25% probability of having received  $A^3$ , and  $F_{G_{3x}} = 0.5$ , so

$$\mathbf{q}_{G_{3x}} = \left[ \frac{1}{4}, \frac{1}{4}, \frac{1}{4}, \frac{1}{8}, \frac{1}{8} \right]. \quad (3)$$

Let  $\mathbf{w}$  be the vector of effects of the five genotypes on logarithmic fitness. The expected log fitness of line  $i$  is then  $\bar{w}_i = \mathbf{q}_i \mathbf{w}^T$ , and the difference in expected fitness between, e.g.  $G_{2x}$  and  $G_{3x}$  is  $(\mathbf{q}_{G_{2x}} - \mathbf{q}_{G_{3x}}) \mathbf{w}^T$  etc. Note that

$$\mathbf{q}_{G_{2x}} - \mathbf{q}_{G_{3x}} = \mathbf{q}_{G_{3x}} - \mathbf{q}_{G_{2s}} = \left[ -\frac{1}{8}, 0, -\frac{1}{8}, \frac{1}{8}, \frac{1}{8} \right], \quad (4)$$

which implies that  $\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}} = \bar{w}_{G_{3x}} - \bar{w}_{G_{2s}}$ . Because at the same time  $F_{G_{2x}} - F_{G_{3x}} = F_{G_{3x}} - F_{G_{2s}}$ , a linear relationship between inbreeding coefficient and fitness is predicted for these three lines. This is valid for arbitrary fitness values, and for any pair of parental genotypes  $P_s$  and  $P_x$ , whether or not the three alleles are unique. Because in the absence of epistasis log fitness is additive across loci, the relationship  $\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}} = \bar{w}_{G_{3x}} - \bar{w}_{G_{2s}}$

holds for multiple loci. Furthermore, it also holds if the parental lines are genetically variable, irrespective of linkage disequilibria. Crosses between such variable population can be broken down into crosses between pairs of individual genotypes; as the eqn 4 holds for each such cross separately, it must hold for their sum.

It should, however, be stressed that the above is a special case. In general, in the absence of epistasis a linear relationship between log fitness and  $F$  is expected only if frequencies of all genotypes are linearly related to  $F$ . This is the case for the three focal lines ( $G_{2x}$ ,  $G_{3x}$  and  $G_{2s}$ ), but the other two lines ( $G_{1x}$  and  $G_{1x}$ ) derived in the pedigree designed by Salathé & Ebert (2003, Fig. 1) do not fit this relationship. This is why the test for epistasis in Salathé & Ebert (2003) was only based on those three crosses, for which the relationship between log fitness and  $F$  was expected to be linear in the absence of epistasis. This was not clearly elucidated in that paper. In contrast to what Trouve *et al.* seem to suggest, whether the two parental lines originate from the same or different local populations is irrelevant for this conclusion.

## Genetic independence of crosses

Trouve *et al.* also question Salathé and Ebert's design on the ground that the lines with different  $F$  were not genetically independent, which may have biased the statistical tests. There are two sources of this genetic nonindependence; we are not sure which Trouve *et al.* allude to, so we discuss them both. First, the tests were based on comparing fitness of lines ( $G_{2x}$ ,  $G_{3x}$  and  $G_{2s}$ ) from the same 'family', i.e. descendant from the same pair of parental lines  $P_s$  and  $P_x$ , replicated across six independent families. This is appropriate: the prediction of linear relationship between  $F$  and fitness applies to lines within a family, related to one another by the specific pedigree, and not to lines occupying the same pedigree position in different families. Deviations from this relationship are thus directly tested by comparing lines within families. The main effect of family (factor 'origin' in the analysis of variance, Table 1 in Salathé & Ebert, 2003) controls for the effect of genetic background (i.e. the identities of alleles  $A^1$ ,  $A^2$  and  $A^3$  in the above model). Secondly, the expectation of linear relationship is based on the probabilities of the three lines ( $G_{2x}$ ,  $G_{3x}$  and  $G_{2s}$ ) carrying specific genotypes (eqns 1–3), assuming that they are sampled independently. However, within each family each line was only represented by a single clonal genotype, and these genotypes were not sampled independently. For example,  $G_{3x}$  was the daughter of  $G_{2x}$ , so  $G_{3x}$  could not be  $A^1 A^3$  if  $G_{2x}$  happened to be  $A^2 A^2$ . As a result, the sampling errors of fitness estimates of those three lines within a family are nonindependent. This indeed in principle violates an assumption of analysis of variance. However, fitness differences observed between those lines are likely to be due to a number of loci, at least some of which would

segregate independently (*D. magna* has 10 pairs of chromosomes). This should average out any potential bias due to nonindependent sampling. Still, to be on the safe side one can take a conservative approach, directly testing the null hypothesis  $(\bar{w}_{G_{2x}} - \bar{w}_{G_{3x}}) - (\bar{w}_{G_{3x}} - \bar{w}_{G_{2x}}) = 0$  with a *t*-test. For this test each family provides a single data point, so the nonindependence of data within a family is not an issue. This test rejects the null hypothesis both in the absence ( $t = 2.78$ ,  $P = 0.039$ , d.f. = 5) and in the presence of parasites ( $t = 6.98$ ,  $P = 0.0009$ , d.f. = 5), confirming the existence of a nonlinear relationship between fitness and *F*.

### What is the relevant reference population?

Inbreeding coefficient and inbreeding depression are defined relative to a reference population, and the choice of the reference population should reflect the biology of the species (Keller & Waller, 2002). While Trouve *et al.* apparently consider the local subpopulation as the reference population, we believe that in our case the entire metapopulation constitutes the biological relevant reference population (Haag *et al.*, 2002). As shown above, the issue of the reference population is irrelevant to the conclusion that the pattern found by Salathé and Ebert implies epistasis. However, these epistatic interactions may have been between alleles originating from different local populations. So the ecological relevance of the pattern found by Salathé and Ebert would depend on how often alleles originating from different local populations meet in the same individual and thus have a chance to express their epistatic interactions. We believe that this happens frequently and therefore the metapopulation is the relevant reference population, a point on which Salathé and Ebert (2003) did not elaborate.

Salathé & Ebert (2003) used material from a highly dynamic metapopulation with average extinction probabilities of nearly 20% per local population per year (Pajunen & Pajunen, 2003). Populations go through extreme founder effects during colonization and suffer from very high drift loads (Ebert *et al.*, 2002). In the most extreme and, to our knowledge, the most frequent cases, one single clone founds a new population. To survive the following winter, members of this clone must sexually produce resting eggs (in this case by a process genetically equivalent to selfing), making the entire population highly inbred ( $F = 0.5$ ) during that next year, even if it has expanded to a large size. Because the populations have, on average, a short time of survival, they do not have time to diverge more than they did through the founder effect: random genetic drift (other than through the founder effect) and the accumulation of mutations do not play a significant role. Thus, over evolutionary time the entire metapopulation shares a common gene pool,

even if at any time local populations may show a pattern of considerable differentiation. Hence, it is justified to regard the metapopulation as the reference population in assessing inbreeding depression. From this perspective, inter-subpopulation crosses (hybrids) are similar to crosses among randomly chosen individuals in a large outcrossing population. The difference between the fitness of inter-subpopulation crosses (hybrid vigour) and crosses within subpopulations is then equivalent to the difference between inbred and outbred lines within a large population.

### Conclusion

To summarize, in the absence of epistasis the relationship in Fig. 4 in Salathé & Ebert (2003) should be linear, irrespective of hybrid vigour. In other words, the marked deviation from linearity cannot be explained without epistasis. The most plausible explanation is synergistic epistasis between recessive deleterious alleles segregating in the metapopulation of *D. magna*, from which the experimental clones originated. Although we believe that Trouve *et al.* (2004) highlight important points relevant to the understanding of epistasis, we do not agree with their suggestion that, for Salathé and Ebert's results, 'hybrid vigour is an explanation as likely as is synergistic epistasis'.

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**Synergistic epistasis and alternative hypotheses**

S. TROUVE, A. DING &amp; J. GOUDET

*Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland**Keywords:*

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**Abstract**

Inbreeding generally results in deleterious shifts in mean fitness. If the fitness response to increasing inbreeding coefficient is non-linear, this suggests a contribution of epistasis to inbreeding depression. In a cross-breeding experiment, Salathé & Ebert (2003, *J. Evol. Biol.* **16**: 976–985) tested and found the presence of this non-linearity in *Daphnia magna*. They argue that epistatic interactions cause this non-linearity. We argue here that their experimental protocol does not allow disentangling the effect of synergistic epistasis from two alternative hypotheses, namely hybrid vigour and statistical non-independence of data.

Salathé & Ebert (2003) recently presented in this journal the results of an experiment on the genetic architecture of inbreeding depression. They examined the relationship between inbreeding level and phenotypic value in the parthenogenetic crustacean *Daphnia magna*. Their main result was a decline of fitness-related characters with the inbreeding coefficient at a greater than linear rate. The authors interpreted this result as evidence that deleterious mutations at different loci interact synergistically.

Our purpose here is not to dispute that synergistic epistasis could explain the results obtained by Salathé & Ebert (2003). Rather, we argue that at least two other processes could lead to the same results, namely the hybrid vigour and the non-independence of data. As hybrid vigour is certainly the most important one, it is considered first.

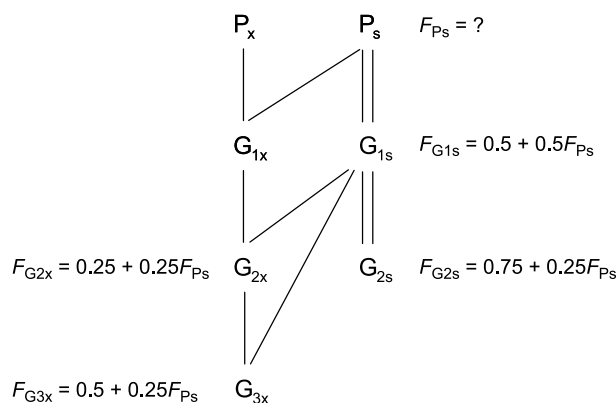
Salathé & Ebert (2003) obtained three classes of increasingly inbred genotypes (Fig. 1). The genotypes with the two lowest levels of inbreeding  $G_{2x}$  ( $F \geq 0.25$ ) and  $G_{3x}$  ( $F \geq 0.5$ ) were derived from a cross between clones from two different subpopulations ( $P_s$  and  $P_x$ ).  $G_{2s}$ , the genotype with the highest inbreeding coefficient ( $F \geq 0.75$ ), was obtained by two generations of selfing of the clone from the  $P_s$  subpopulation. Therefore,  $G_{2s}$  genotype differs from  $G_{2x}$  and  $G_{3x}$  not only by its inbreeding coefficient, but also by its genetic background:

*Correspondence:* S. Trouvé, Department of Ecology and Evolution, Biology Building, University of Lausanne, 1015 Lausanne, Switzerland.

Tel.: 00 41 21 692 42 44; fax: 00 41 21 692 42 65;  
e-mail: sandrine.trouve@ie-zea.unil.ch

$G_{2x}$  and  $G_{3x}$  are constituted by a mix between two genomes ( $P_s$  and  $P_x$ ), whereas  $G_{2s}$  is made of genes from  $P_s$  only. In this context  $G_{2x}$  and  $G_{3x}$  could benefit from hybrid vigour whereas  $G_{2s}$  cannot. Enhanced fitness is well known to occur after several types of crosses between (sub)populations, including  $F_1$ , backcrosses,  $F_2$ , etc. (Lynch, 1991; Falconer & Mackay, 1996; Burke & Arnold, 2001 and references therein). And indeed, Ebert *et al.* (2002) have recently demonstrated that very strong hybrid vigour is present in populations of *D. magna* from southern Finland, the area where the parents of the crosses discussed here are coming from.

We therefore argue that the non-linear response of fitness observed could result from a fitness advantage of



**Fig. 1** Experimental protocol used in Salathé & Ebert (2003) and inbreeding coefficient of the different genotypes. Figure modified from Salathé & Ebert (2003).

$G_{2x}$  and  $G_{3x}$  due to hybrid vigour. This genetic process is usually attributed to different deleterious mutations randomly fixed in the different populations (a phenomenon termed drift load: Whitlock *et al.*, 2000; see Keller & Waller, 2002 for review) and to the net masking of their deleterious effects in among-population crosses. It could be argued that hybrid vigour is simply inbreeding depression in reverse. But additive by additive epistasis can contribute to hybrid vigour (Lynch, 1991), while inbreeding depression only occurs if some form of dominance (with or without epistasis) is present (Lynch & Walsh, 1998, p. 258). Therefore inbreeding depression and hybrid vigour can have a different genetic basis and cannot be considered as the same phenomenon.

A way to test the existence of hybrid vigour in the experimental design of Salathé & Ebert (2003) would be to examine the effects of within and between-subpopulation crosses on the relative performance of the progeny. These comparisons of fitness would need to be performed on progeny with equivalent inbreeding coefficients in the two types of crosses. If offspring from between-subpopulation crosses show higher fitness compared with offspring issued from crosses within subpopulations, this could confirm that the mix between two genomes may enhance fitness in  $G_{2x}$  and  $G_{3x}$  genotypes.

Another explanation for the results observed by Salathé & Ebert (2003) has to do with the non-independence of data (Lynch & Walsh, 1998, p. 262). In the protocol they used,  $G_{3x}$  is a genotype directly derived from  $G_{2x}$ . Indeed a backcross between  $G_{2x}$  and  $G_{1s}$  gave  $G_{3x}$ . As these two data points ( $G_{2x}$  and  $G_{3x}$ ) are based on individuals that are descendants of each other, their fitness is therefore not independent, and this might partly explain their similar values. An associated issue is that the non-independence of data creates a statistical problem: a basic assumption underlying ANOVA (the test used in their study) is violated.

Lynch & Walsh (1998, p. 265) suggest directions for alleviating this problem of non-independence of data. The general idea is based on crosses of various classes of

relatives to obtain simultaneously independent lines inbred to differing degrees. Furthermore, if the crosses are performed within (sub)populations, one can avoid the confounding effect of hybrid vigour.

In conclusion, we do not dispute that synergistic epistasis is a potential explanation for the non-linearity in the data observed in Salathé & Ebert (2003), but in the light of the previous work carried by Ebert *et al.* (2002), we feel that hybrid vigour is an explanation as likely as is synergistic epistasis.

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