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Risk-spreading by mating multiply is plausible and requires empirical attention

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Manuscripts

1 **Risk-spreading by mating multiply is plausible and requires**
2 **empirical attention**

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16 We appreciate Henshaw and Holman's [1] (henceforth HH) Comment regarding our original
17 article [2]. We understand the points made by HH but we have reservations about their
18 applicability to our data, as explained below. Before addressing the specific details in HH's
19 commentary, however, it is important to note that the substance of their article deals with
20 technical aspects of our statistical analysis, not the underlying theoretical framework or the
21 empirical design employed in our study. Indeed, we are happy to see that HH acknowledge
22 that our work offers a valid proof-of-principle approach for studying the role that bet-
23 hedging plays in determining the benefits of multiple mating in isolation from other factors,
24 namely sexually selected mechanisms. Indeed, that was the main motivation of our study,
25 rather than to specifically document the benefits of bet-hedging and sexual selection in the
26 subject species.

27
28 To briefly recap, our study revisited the concept of bet-hedging in the context of explaining
29 female multiple mating, identified common misunderstandings surrounding its
30 interpretation, and offered a novel experimental approach to test for its existence. Our

31 study system, the sea urchin *Heliocidaris erythrogramma armigera*, offers remarkable levels
32 of experimental control via *in vitro* fertilizations, thus enabling us to analyse temporal
33 (geometric mean) fitness among females assigned *simultaneously* to a polyandrous and
34 monandrous mating strategy. In this way, we controlled for the effects of female genotype
35 and maternal effects on fitness outcomes, whilst separating ‘pure’ bet-hedging effects from
36 sexually selected paternity-biasing mechanisms by manipulating the fertilization conditions
37 (presence or absence of competition among the gametes of different males). The underlying
38 question addressed in our paper was to determine whether the intergenerational fitness of
39 females is higher when they follow a strategy that increases mate sampling (i.e., polyandry)
40 compared to a non-bet hedger strategy (monandry). By simulating reproductive bouts across
41 generations, we uncovered the potential for bet-hedging, in addition to paternity-biasing
42 mechanisms (e.g. sexual selection), to provide increases in fitness for multiply mated
43 females.

44

45 HH’s first comment suggests that technically, Gillespie’s measure may provide a more
46 appropriate fitness measures for our data. We appreciate the suggestion here, but note that
47 Gillespie’s measure hardly deviates from our use of geometric mean fitness, which can be
48 understood as a valid proxy of intergenerational fitness (e.g., compare, for each trait, the
49 data in the first and third rows in the first column of HH’s Table 1). Second, HH acknowledge
50 that the analysis of alternating environments of the form ABA and BAB requires complex
51 analysis. However, in the event, HH do not carry out such analysis. Instead, they apply
52 bootstrapping to estimate confidence intervals (CIs) on mean effect sizes using our measure
53 of evolutionary fitness (geometric mean) and Gillespie’s measure. If the main objective of
54 HH’s comment is to provide true CIs around bet-hedging effects in our data (see below),

55 then we assume that implicit in their decision to use our or Gillespie's measure is the fact
56 that these measures indeed yield estimates of intergenerational fitness that are good
57 approximations of those that would be obtained with the more complex analysis. This fact,
58 together with the very similar values for the delta statistics provided in the first column of
59 HH's Table 1 independently of the method used, lead us to believe that the technical points
60 made by HH on our use of geometric mean fitness can distract the reader from focusing on
61 the question that matters: Is there scope for bet-hedging benefits when females mate with
62 multiple males?

63

64 HH's comment further suggests that the original methods in our analyses underestimate the
65 bounds in the fitness differentials that we found empirically. In this respect, it is important to
66 note that our proposed design yields data on a simulated multi-generational scale and that
67 the different generations could be arranged in different orders. As such, there was no single
68 fixed dataset generating a single effect size (difference between the geometric mean fitness
69 of a polyandrous strategy and a monandrous strategy), but a multitude of potential
70 outcomes (effect sizes) depending on the ordering of generations. Our original analysis
71 therefore included the precaution of reshuffling the order of the generations to provide a
72 distribution of effect sizes reflecting the 'universe' of potential effect sizes that could be
73 obtained with the real data. HH are aware of this fact but suggest that bootstrapping would
74 have been useful for estimating the uncertainty around our effect size estimates, and that
75 null hypothesis testing could have been carried out by generating a null distribution. We
76 respond to each of these points in turn:

77

78 To address the point regarding confidence intervals for our effect sizes, we agree with HH
79 about the benefits of using bootstrapping but stress that the aim of our method was to
80 address the uncertainty arising from the fact that effect sizes could be calculated from
81 thousands of equally plausible combinations of real data obtained within the experiments.
82 By contrast, the focus of bootstrapping lies in addressing the uncertainty resulting from the
83 stochastic nature of sampling. These sources of uncertainty are different, but both are
84 important and complementary. Our paper did not provide *true* confidence intervals on a
85 given, fixed, outcome, but instead provided all possible outcomes. We regret not to have
86 made this point clearer in our study and are grateful for the opportunity of clarifying this
87 here. We agree with HH that to approximate *true* confidence intervals on any given
88 estimate, bootstrapping is a more appropriate method. The key point to note, however, is
89 that in our study there was not a single particular estimate, but a full range of them as a
90 result of multiple equally plausible outcomes. Nevertheless, HH apply bootstrapping as if
91 there was indeed a single estimate, but they source the bootstrapped data sets not on a
92 single “real” data set but on multiple datasets arising from the reshuffling of generations. In
93 our view, bootstrapping would be more appropriate if one of the myriad of potential re-
94 arrangements of data yielding an effect size similar to the mean effect size obtained with the
95 re-ordering of generations was selected. Bootstrapping could then be performed on that
96 particular dataset. One could go further and repeat this several times with other datasets to
97 generate a mean value (or a value close enough to the mean value), and the 95% CIs could
98 then be averaged. The same procedure could be employed for other effect sizes on top of
99 the mean effect size.

100

101 On this point of HH's generation of effect size confidence intervals we are troubled by HH's
102 statement that "*Because individual females and males appear multiple times in the original*
103 *experiment, this procedure will tend to underestimate the true population variances, and*
104 *hence the strength of bet-hedging effects. We nevertheless believe this pseudoreplication is*
105 *unlikely to affect the results strongly*". We want to clarify that this comment about
106 pseudoreplication applies to HH's analysis, not to our experiment or analyses, as the above
107 statement may seem to imply to the reader. Females in our experiment represent distinct
108 genotypes that are assayed across three generations each (this is the purpose of the design);
109 our analysis takes this fact into account and estimates intergenerational fitness accordingly
110 [2]. As for males, they are not used across generations or across blocks (females). The point
111 was to mimic females that are sampling (either monandrously or polyandrously) from a
112 series of available males in each generation; within each block and generation a male was
113 shared between the two mating strategies (the male mated to the monogamous strategist),
114 but the analysis takes into account the paired structure of the data (see [2] and associated
115 ESM).

116

117 An additional point raised in HH's commentary was the suggestion to test observed fitness
118 differentials against a null distribution that assumes no difference in geometric mean fitness
119 between monandrous and polyandrous treatments. We agree with the premise of this
120 suggestion, but stress that it is limited by the same problem described above. HH focus on
121 just one of a myriad of potential outcomes - one that yields the mean of the distribution of
122 effect sizes in our original study but approximates the probability to obtain this statistic by
123 using the whole range of data sets that our design provides. This method superimposes the
124 re-arranging of treatments for null hypothesis testing upon the re-arranging of generations,

125 and this is bound to produce wide CIs. Here we outline an alternative way to test single point
126 outcomes, including the mean of the distribution of effect sizes provided in our original
127 study. First, we suggest extracting a single data set that provides the mean effect size (or
128 very approximate value). We then suggest randomizing the treatments in this data set to
129 obtain the null hypothesis distribution from which to get the p value for obtaining an effect
130 at least as large as the mean effect seen empirically (i.e., using the re-ordering of
131 generations). This could be performed several times on numerous data sets that provide the
132 mean effect size value (or values close to this), and one could calculate the mean p , if one
133 wanted to get a more precise p value than that obtained with a single data set. This protocol
134 could be employed to test other effect sizes on top of the mean effect size.

135

136 Despite the limitations of HH's approach outlined above, HH acknowledge that large effects
137 of bet-hedging (Experiment 1) are still plausible. Indeed, the 95% CIs calculated by HH
138 suggest caution in rejecting the hypothesis for the absence of bet-hedging effects on
139 offspring viability in environment A, supporting our original conclusions surrounding the
140 potential of bet-hedging to bring benefits to multiply mated females.

141

142 We set up high levels of replication for the units of analysis within each block by setting 18
143 independent batches of eggs per female, and measured thousands of offspring to reduce
144 sampling variation around the measures of female fitness. This, however, compromised the
145 number of individual female genotypes inspected, which in turn inevitably leads to an
146 increase in the uncertainty in our conclusions at the population level. We full concur with HH
147 that higher levels of replication will be ideal in future empirical tests of polyandry via bet-
148 hedging, but emphasize that the main objective of our study was to raise awareness among

149 researchers about empirical ways to test bet-hedging ideas, rather than to carry out a
150 definitive test of these ideas on sea urchins.

151

152 In short, we welcome HH's critique because it generates an interesting debate about the
153 ways to assess significance in complex designs similar to the design that we propose in our
154 original contribution. HH's commentary, in conjunction with our original study and the
155 present reply may be useful for improving our capacity to test the theory in the future. After
156 HH's remarks we reaffirm that the main messages and conclusions in our original study
157 remain valid. To summarize:

158

159 (1) We provide a tractable and innovative experimental approach for addressing bet-hedging
160 theory.

161 (2) Our empirical results suggest that the evolution of polyandry via bet-hedging should not
162 be overlooked. There is scope for a multiple-mating strategy to provide intergenerational
163 increases in fitness due to benefits associated with risk spreading.

164 (3) Our results also show that sexual selection (arising from deterministic paternity biasing
165 mechanisms) can augment the potential benefits of multiple mating attributable to risk
166 spreading mechanisms (which do not require reliable mate assessment).

167 (4) Collectively, our findings call for an increased effort in undertaking empirical tests of bet-
168 hedging theory in ecology and evolution.

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176

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