Letter to Editor

Testing the level of ant activity associated with quorum sensing: An empirical approach leading to the establishment and test of a null-model (response to the comment of Richardson et al.)

ABSTRACT

In a paper in this journal (Nouvellet et al., 2010), we presented results from experiments on the behaviour of the Pharaoh's ant, *Monomorium pharaonis*, along with a substantial statistical and theoretical analysis of the results. In a minor part of our paper, we compared our results with the related work of Richardson et al. (2010a). These authors have subsequently commented on our interpretation of their work (Richardson et al., 2011). In this Letter we respond to the comments of Richardson et al. (2011), and give detailed arguments why we stand by our original conclusions.

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1. Introduction

Reasoned debate between scientists is one way in which science makes progress, and our paper (Nouvellet et al., 2010) has generated such an exchange. In particular, Richardson et al. (2011) made comments in this Journal on a minor part of our paper (Section 8 and Appendix C). Here we address their comments.

The focus of our paper was on the level of activity of ants, as a function of density of conspecifics. We located a bridge-like structure very close to an ant nest (see Fig. 1 of Nouvellet et al., 2010). The ants presumably encountered the bridge in a stochastic fashion, when undergoing random exploration. Ant movement onto the bridge was therefore taken as a proxy for ant activity in the area immediately outside the nest. We considered two situations: one, where the number of ants is constant; the other, where the number of ants steadily declines. When the total number of ants in the nest/nesting area was constant, we found a stationary exponential distribution of time intervals between adjacent ants leaving the bridge, as anticipated. This has the natural interpretation that the ants were acting as independent agents. By contrast, we had expected to find some effect on the activity of the ants as their mean density in the nest/nesting area steadily decreased, ultimately to ~10% of its initial value. However, our mathematical modelling and statistical analysis of the data showed that a simple rescaling of time, that took account of the number of ants remaining in the nest/nesting area, was sufficient to explain all observations; we thus found strong evidence that the ants did not modify their behaviour in any significant way as their number was decreasing, and they continued to act independently of each other. This was unanticipated because, as stated in our paper, there are many examples of unicellular and multicellular organisms responding behaviourally to the density of their conspecifics.

A key point to note is that when replacement was not allowed in our experiments, the resulting data were not homogeneous in time.[1] In this sense it shares the feature of being heterogeneous in

time, like the log-Poisson process advocated by Richardson et al. (2010a). In the resulting model of our data and the log-Poisson process, expected time intervals between individuals' exiting have the feature of growing with time (exponentially in our case, linearly in the case of Richardson et al.).

2. Points of disagreement

Let us now come on to specific points of disagreement with Richardson et al. (2011). We use the numbering scheme adopted in their Letter.

(1) Nouvellet et al. (2010) investigated ants leaving "their nesting area (i.e., the brood chamber and its immediate vicinity)" while Richardson et al. (2010a) investigated ants that "left the nest". Thus there are clearly stated differences between the experiments, but also substantial similarities that allow comparison. *As far as activity is concerned*, experiments where ants irreversibly fall off a bridge-like structure which they encounter (Nouvellet et al., 2010) are comparable with experiments where the ants that are leaving the nest are irreversibly expelled with a puff of air (Richardson et al., 2010a). We note that Richardson et al. (2011) attribute differences in the conclusions of their work and our own work, to "indirect interactions", including the use of pheromone trails by Pharaoh's ants (Jackson et al., 2006). This appears to stem from two misconceptions of Richardson et al. (2011) concerning our experimental setup.

(i) We reject the notion that our set-up was artificial and excluded indirect interactions; our nest/nesting area had been established for several months prior to the experiments, which is more than enough time for pheromone trails and other "indirect interactions" between ants to be established. The introduction of a bridge-like structure was the only modification to the ants' environment.

(ii) Contrary to the view of Richardson et al. we note that the pheromone trails of the Pharaoh's ants in our experiments can vary on a time scale of tens of minutes (Nouvellet et al., 2009), which is a much shorter time than the duration of the experiments we carried out. Such trails, and their dynamic nature, may thus have been highly informative to the ants in our experiments.

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[1] It is the scaling of time differences, that we carried out on our data, that converted time-heterogeneous data to time-homogenous data.

(2) Richardson et al. (2011) misrepresent matters by writing that we "attempt to discredit" their conclusions. We simply indicated that, on the basis of the evidence they produced, we were unconvinced by their conclusions. We remain so; see below.

(2a) We certainly do not argue "that because [we] do not find compatibility with a log-Poisson process, [Richardson et al., 2010a] should not find it either". Rather we produced very strong evidence that *our* experiments are incompatible with a log-Poisson process. We proceeded to show that, using the graphical approach of Richardson et al. (2010a), one could be misled into thinking a log-Poisson process was in place. We further stated, very clearly in Section 8 of our paper, an objective way to discriminate between the two processes.

(2b) Firstly, to dispel any doubts in the reader's mind, we note the figures plotted in the current online version and the current print version of our paper, including Fig. 11, are the correct versions. Secondly, plotting the figure using logarithms to base 10 or using natural logarithms would give curves of *exactly the same shape*; $\log_{10}x$ is directly proportional to $\log_e x$; thus our conclusions remain that visual inspection of our Fig. 11a indicates that it is quite similar to some of the plots in Fig. 3 of Richardson et al. (2010a), and the same is true for our Figs. 11b and 4 in Richardson et al. (2010a). We add that contrary to the claim made by Richardson et al. (2011), our data are linear over a range in excess of one order of magnitude of times, and not the 0.2–0.4 orders of magnitude stated in their comment. An important question that Richardson et al. do not address, is: how many orders of magnitude should a log-linear relationship hold before it is accepted by visual inspection? (Visual inspection was part of the method adopted in Richardson et al. (2010a)).

We presented Fig. 11 merely as a way of comparison, and to point out that a plot of the survivorship function is a *weak* method to discriminate the two processes. However, we continue to argue that the definitive analysis of exiting data should be an explicit test of the actual distribution. In our non-replacement experiments, the exponential nature of the distribution should be (and was) tested on *scaled* time intervals, while in the corresponding experiments by Richardson et al. the log Poisson distribution should be (but was not) tested on the difference of logarithms of the times of exiting.

(2c and d) Reading Richardson et al. (2010a), we were under the impression that they directly tested the exponential nature of their distribution using the difference of the logarithms of the times of exiting. This was based on our misreading of their Table S1 and its cryptic caption. This mistake on our part was based on our assumption that Richardson et al. (2010a) actually tested the key assumption of their work: that differences of logarithms of the exiting times are exponentially distributed. It thus appears that this fundamental assumption was not tested.

Consequently the strongest evidence for a record dynamics process is that the survivorship function, associated with differences of logarithms of exiting times, is linear when plotted on a log scale. This was tested using linear regression. Additionally, the normality of residuals was checked. The linear regression in itself is of little relevance since, by definition, a survivorship function is a monotonically decreasing function (see our Fig. 11a) and hence the null hypothesis (that the survivorship shows *no* specific trend) would be rejected regardless of the underlying distribution of differences of the logarithms of exiting times. Thus normality of the residuals is the essential component of their analysis. However, since it would have been possible to directly test the nature of the distribution, the statistical methods adopted by Richardson et al. (2010a) appear to be convoluted and rather weak.

Referring again to Table S1 of Richardson et al. (2010a), we accept the comment of Richardson et al. (2011) that a meta-analysis tests heterogeneity of *p*-values rather than gives an overall *p*-value. However, the fact still remains that 5 out of 13 experiments (in the removal case) and 3 out of 7 experiments (in the non-removal case)

appear to show deviations from a linear relationship. We thus remain unconvinced by the claim of Richardson et al. (2010a) that their data provide evidence of a log-Poisson process. We restate our comment that the test they apply to their data to demonstrate a log-Poisson process is rather weak and that a convincing test would be to show that differences in the logarithms of exiting times are indeed exponentially distributed.

Finally, in their comment, Richardson et al. (2011) mention further evidence for a record dynamics type of process that was drawn from a recent publication (Richardson et al., 2010b). Their new study does show a very clear pattern of a decreasing per capita rate of nest exiting with time. Interestingly, this conclusion is in clear contrast with our study, and confirms that, while the experimental set-ups are broadly comparable, some differences remain. However, we see no strong evidence to favour a record dynamics process for two reasons. First, they reject a heterogeneous Poisson process on a finite population based on *visual inspection*. It would be better to test the record dynamics hypothesis against the null hypothesis of a heterogeneous Poisson process on a finite population (possibly using an AIC method). This problem is very similar to that exposed in Edwards et al. (2007). Furthermore, a reading of the paper (Richardson et al., 2010b) reveals that again the *distribution* of the difference of the logarithms of exiting times is not tested. Finally, even if behaviour *consistent* with record dynamics is found, this alone is not compelling. Without an explicit model for the behaviour of the ants, adopting record dynamics is akin to picking a stochastic process from the shelf. One should be particularly careful in doing this as there exist an infinity of stochastic processes that can be compatible with the observed behaviour.

3. Conclusions

The work of Nouvellet et al. (2010) provided very strong evidence, in a given set-up and for a given species of ants, that very simple, independent ant activity characterises the dynamics. By contrast, Richardson et al. (2010a, b, 2011) have made arguments that in their set-up, for a different species of ant, a more complex process is occurring. The onus is, and remains, on them to compellingly demonstrate this process, rather than being satisfied with the substantially weaker statements of compatibility with their experiments. We strongly believe that if a more complex process is to be demonstrated, it should be statistically tested against the proper null hypothesis.

We began this Letter by talking about the progress of science. It is apparent that Richardson et al. (2010b) do report clear differences in behaviour compared with the results of Nouvellet et al. (2010). If the results of Richardson et al. (2010b) are statistically established to be log Poisson, then the interesting scientific question to be addressed is: what does it take to change the dynamics from Poisson-like to log-Poisson-like? Theoretical work has already been initiated on this issue (Sibani and Christiansen, 2011).

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