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Forum

Reassessment confirms motivational trade-offs and modulation of nociception in bumble bees

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We thank Read and Nityananda (2026) for critically engaging with our paper, Gibbons, Versace et al. (2022), ‘Motivational trade-offs and modulation of nociception in bumblebees’. In this response, we address each of their concerns. We clarify that our main finding, the significant negative interaction between the response to aversive heat and lower relative sucrose concentration, is well supported. Even by Read and Nityananda's own standards, finding such an interaction while using our design is evidence of flexible motivational trade-off behaviour that cannot be explained by simple signal detection theory. Overall, the reassessment based on Read and Nityananda's (2026) critique has reinforced our case: our original analysis is confirmed as the most robust and appropriate for the data, and our conclusion that bees perform flexible motivational trade-off behaviour remains strongly supported.

WHAT MAKES A TRADE-OFF COMPATIBLE WITH POTENTIAL SENTIENCE?

We welcome Read and Nityananda's (2026) theoretical analysis of what constitutes a ‘good’ motivational trade-off. As Brown and Birch (2025) have also argued, refining the design of motivational trade-off experiments is essential for advancing sentience research, and we see this as a constructive step forward.

Read and Nityananda emphasize two criteria for a motivational trade-off to be relevant to detect sentience: (1) the trade-off must rely on internal representations rather than direct sensory input and (2) there must be a statistically significant interaction between the responses to the stimuli.

Our work (Gibbons, Crump et al., 2022; Gibbons, Versace et al., 2022) meets these criteria: bees relied on internal representations to implement the trade-off, the trade-off was confirmed when bees used memory alone (Gibbons, Crump et al., 2022), and we found a significant negative interaction. Read and Nityananda (2026) agree with the internal representations required by our design, but dispute our finding of a significant interaction. We note

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that their criticism targets a model that we did not use (see ‘Clarification of statistical approach’ below), while our previous results stand.

Criterion 1: The Trade-off Must Rely on Internal Representations Rather Than Direct Sensory Input

Read and Nityananda (2026) explicitly acknowledge that our design fulfils this requirement, since bees were required to form internal representations of reward based on learnt feeder colour. This differentiates our findings from their hard-wired microbe example: ‘This behaviour cannot be fully hard-wired, because the bees made choices based on the colour of the feeder without sensing the sucrose directly. The bees must have been generating their reward signal via an internal representation of the reward associated with each colour’.

Further supporting this conclusion, bees showed significantly fewer events where they landed on a feeder but did not feed in the last foraging bout than the first. This demonstrates that the bees’ decision to feed from a particular feeder was not based on direct simultaneous sensation of the stimuli, but on an internal representation of the feeders.

We further confirmed this in a separate experiment with an independent, unreinforced memory test (described in Gibbons, Crump et al., 2022; Fig. 1). Bees were trained with feeders offering either 10% versus 40% sucrose, or 40% versus 40% sucrose, across two phases where the 40% feeder was first unheated and then heated. During the unreinforced test with both feeders being unheated and containing only water, bees’ preferences mirrored the overall heated feeder preferences observed both in this experiment (10% versus 40%: $V = 3.5$, $P = 1$; 40% versus 40%: $V = 3.5$, $P = 1$; see Fig. 1) and in our original experiment (10% versus 40%: $W = 40$, $P = 0.0726$; 40% versus 40%: $W = 28.5$, $P = 0.911$; see Fig. 1). Therefore, bees continued to avoid the feeder type that had previously been heated when rewards were equal, but preferred the heated feeder when it had been associated with relatively higher sucrose concentrations.

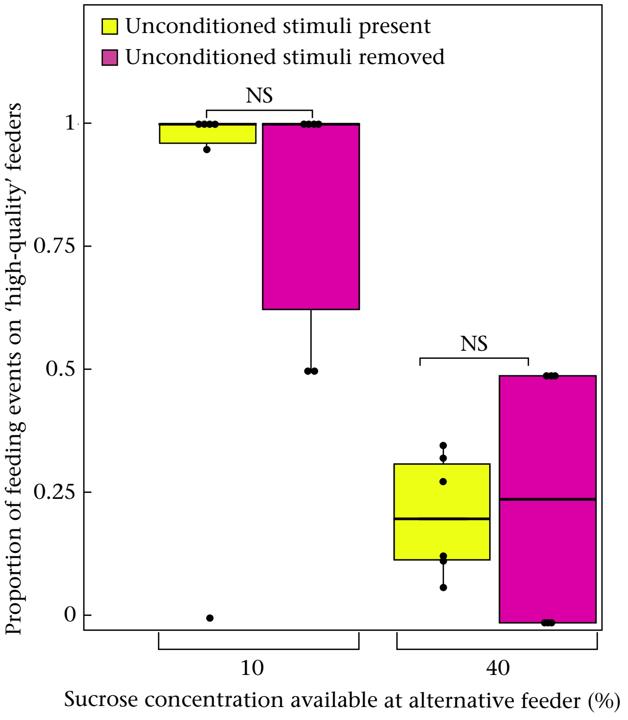

Figure 1. Memory-based motivational trade-off between sucrose concentration and aversive heat. Bees were offered two feeder types: one that always contained 40% sucrose solution (the high-quality feeder), and one, the alternative feeder, that contained either 10% or 40% sucrose solution (depending on the assigned condition). Each bee was exposed to two temperature phases, first where both feeders were unheated, then where only the high-quality feeder was heated to an aversive temperature (‘unconditioned stimuli present’). Subsequently, bees were given a memory test after both temperature phases, where sham feeders with the same colour and position were presented, but without sucrose or heat (‘unconditioned stimuli removed’). Box plots show the proportion of feeding events at the high-quality feeder in the two sucrose conditions. Individual data points (mean proportion per bee) are overlaid on each box. The horizontal line within each box shows the median, the upper and lower horizontal lines the interquartile range and whiskers represent 1.5 × interquartile range.

Criterion 2: There Must Be a Statistically Significant Interaction Between the Responses to the Stimuli

Read and Nityananda (2026) argue that ‘the trade-off of heat and sucrose does not in itself represent new evidence about bee sentience’. We agree, and this was the rationale behind our experimental design, stated in the original paper: ‘We expanded on the motivational trade-off paradigm by ensuring that the trade-off relied on conditioned cues associated with the motivational stimuli, rather than direct sensory experience of the stimuli themselves’.

We recognized that a trade-off between heat avoidance and sucrose preference is insufficient if the relative values are fixed, and that context-dependent flexibility is required, which is demonstrated by a significant negative interaction. Our data revealed exactly such an interaction.

While Read and Nityananda (2026) dispute our finding of a significant interaction, they are clear that finding one in our set-up would be meaningful: ‘An interaction term also rules out the simple decision model presented in Fig. 1c and d […] Thus, a significant interaction term could be viewed as showing bees are making the sort of flexible motivational trade-off taken as evidence for sentience’.

This is why our significant interaction led us to conclude that the bees exhibited a flexible motivational trade-off. Such a memory-based value representation must occur in the central nervous system.

Ultimately, when considering our actual model, the negative significant interaction found, and the memory experiment, our data cannot be fully explained by simple signal detection theory, as demonstrated by Read and Nityananda's (2026) analysis. Their formalization actually shows why, when an interaction term between internal representations of different stimuli is found, simple mechanistic explanations can be ruled out (such as those invoked in microbial behaviour). Hence, Read and Nityananda (2026) usefully clarify the conditions under which a trade-off can be considered potential evidence of sentience: conditions that our work satisfies.

RATIONALE BEHIND OUR INCLUSION CRITERION

Read and Nityananda (2026) criticize our application of the inclusion criterion. Here, we clarify why these objections misrepresent our methodology and why the criterion is essential.

Criticism 1: ‘The Inclusion Criterion Removed Nearly a Third of Bees’

This is incorrect. We excluded nine out of 41 bees (ca. 22%), which is not ‘nearly a third’. More importantly, these exclusions were justified: we only kept bees that showed a statistically significant preference for high-sucrose feeders (or were at chance in the equal-sucrose condition) in the unheated (control) conditions.

This was necessary to attribute any subsequent changes in preference to the manipulation of temperature, rather than to a failure to discriminate between sucrose concentrations. Without this criterion, we would have risked including bees that had not learnt the difference between heat levels or between sucrose concentrations, and thus could not express a trade-off even if they had the capacity. Read and Nityananda's (2026) approach would have, therefore, increased the risk of a type II error, masking evidence of motivational trade-off behaviour.

Criticism 2: 'The Criterion Was Not Applied to the Heated or Equal-Sucrose Conditions'

This is also incorrect. The criterion was applied to the equal-sucrose condition, although no bees failed it. It was also applied to the heated condition, based on performance in the corresponding unheated (control) condition. Because the unheated and heated trials differed only in the temperature manipulation, our variable of interest, applying the criterion after the manipulation would have introduced post hoc bias and compromised the validity of the analysis.

CLARIFICATION OF OUR STATISTICAL APPROACH

Read and Nityananda (2026) state that our conclusions hinge on a model with random effects specified as:

(1 + Temperature | colony/subject)

This is not the model we used. We note that the Supporting Information of our study correctly described the model, while the main text mistakenly reported the output of the unsimplified version, so $P = 0.039$ was reported instead of the correct $P = 0.033$.

Our final, actual model (after simplification) excluded colony as a random effect and was specified as:

(1 + Temperature | subject)

This correction does not change the significance of the results ($P < 0.05$), and we are grateful for the opportunity to correct this error (correction available at https://doi.org/10.1073/pnas.2522767122).

Thus, Read and Nityananda's (2026) statistical criticisms are directed at a model that we did not use. When assessed against our actual model, none of their objections hold.

Statistical Criticism 1: 'The Model Should be Simplified TTo (1 | Subject)'

This is incorrect. Our model fits the data better than the proposed simplified model on all recommended statistical tests, including the Akaike information criterion (AIC: Akaike, 1998); Bayesian Information Criterion (Schwarz, 1978); deviance, log-likelihood and likelihood ratio tests (LRTs: Bolker et al., 2009; see Table 1).

Statistical Criticism 2: 'The Significant Interaction Relies on excluding Bees'

This is incorrect. Read and Nityananda (2026) reported no significant interaction when running their simplified model with all 41 bees, with an AIC score of 473.6. Using our actual model with the same data set, the interaction remains significant ($Z = -2.084$, $P = 0.037$) and the model fit is greatly improved (AIC = 386.95).

Statistical Criticism 3: 'The Significant Interaction Relies on Bee Number 40'

This is incorrect. Read and Nityananda (2026) found that removing bee number 40 abolished the positive significant interaction in their model, and the negative significant interaction in the model they thought we used. Using our actual model, the interaction remains significant after removing bee number 40 ($Z = -2.042$, $P = 0.041$).

Statistical Criticism 4: 'The Singularity Warning Invalidates the Model'

This is incorrect. While they simplified the model to eliminate a singularity warning, best practice dictates that singularity warnings are not, by themselves, grounds for removing important model components (Barr et al., 2013; Bates et al., 2015; Matuschek et al., 2017). Model reduction should be guided by likelihood ratio tests and theoretical considerations. It is also worth noting that, using the current version of R (Version 4.5.1), our model does not produce a singularity warning, unlike in older versions.

In our study, backward simplification confirmed that removing the colony slope was justified ($\chi^2_3 = 0.919$, $P = 0.821$), while removing the slope for temperature was not ($\chi^2_2 = 87.224$, $P < 0.001$). This confirms that the random slope for temperature is necessary and should not be removed. Removing the slope (as Read & Nityananda, 2026 suggest) assumes that, although bees may differ in their baseline preferences, they all respond identically to the temperature change. This assumption is both contradicted by our data, which show substantial interindividual variation in temperature response (variance = 7.02) compared to baseline preference (variance = 0.013), and biologically implausible, given well-documented individual variability in bee behaviour (Muller & Chittka, 2012; Tait et al., 2019). Therefore, the random slope for temperature should not be removed.

The singularity warning in our model arose because the model cannot reliably distinguish between the baseline differences and the temperature response differences, reflected in the negative correlation (−1.00) and the near-zero intercept variance (0.013) compared to substantial slope variance (7.02). This is a common

Table 1
The output of model comparison tests comparing our model and Read and Nityananda's (2026) model

Test	Our model: (1 + Temperature | subject)	Read and Nityananda's suggested model: (1 | subject)	Better model based on test
Akaike information criterion[1]	230.29	313.52	Our model
Bayesian information criterion[2]	245.40	324.31	Our model
Log-likelihood[3]	-108.15	-151.76	Our model
Deviance[4]	216.29	303.52	Our model
Likelihood ratio test[5]	$P < 0.001$		Our model

[1] Akaike (1998); [2] Schwarz (1978); [3,4,5]Bolker et al. (2009).

computational outcome in ecological and behavioural data (Harrison et al., 2018).

To confirm the cause of the singularity, we ran two models. The first model removed the correlation term but retained the slope, essentially treating the intercept and slope as independent, specified as:

$(1 + \text{Temperature} \,||\, \text{subject})$

Using this model, the singularity warning behaviour varied across software versions.

The second model removed the random intercept but retained the slope, specified as:

$(0 + \text{Temperature} \mid \text{subject})$

Using this model, the singularity warning disappeared.

Importantly, the fixed effects results were unchanged for both of these models compared to our model, and performed significantly better than Read and Nitayananda's proposed slope-free model ($\chi^2 = 87.224$, $P < 0.001$). This confirms that the warning reflected correlation structure, not model invalidity, and that the inclusion of the random slope is statistically necessary.

To summarize, in their attempt to remove the singularity warning, Read and Nityananda (2026) excluded the statistically necessary (LRT: $P < 0.001$) random slope for temperature. In doing so, they made an overly simplistic model that performs worse than our model on every tested metric of model fit, is sensitive to the removal of one bee, and does not account for core aspects of our experimental design. Our model remains the most appropriate and robust method for analysing these data and, thus, our negative significant interaction is well supported.

CONCLUSION

Read and Nityananda's (2026) critique targets a model that we did not use. When their claims are assessed against the correct model described in our Supporting Information (Gibbons, Crump et al., 2022), none of their statistical criticisms hold. This exchange, however, has allowed us to correct a reporting error ($P = 0.039$ instead of the correct $P = 0.033$), and to explain the theoretical and statistical robustness of our approach. Importantly, Read and Nityananda (2026) acknowledge that finding a negative significant interaction in our experiment could constitute evidence relevant to insect sentience. While their proposed model does not demonstrate such an interaction, it also performs worse than our model on all standard model fit metrics, fails to account for the repeated-measures design, underestimates individual variability and is sensitive to the removal of one subject. By contrast, our original analysis remains valid, robust and statistically superior. The significant interaction we observed in Gibbons, Versace et al., 2022 is still supported, and is, in fact, slightly strengthened.

This exchange represents a constructive contribution to the broader dialogue on insect sentience. Motivational trade-offs alone do not confirm sentience, but they form one important line of evidence. Our findings add to a growing literature on the possibility of emotion-like states in bees, consistent with, although not definitive proof of, sentience, including play-like behaviour (Galpayage Dona et al., 2022), responses suggestive of pain-like experiences (Gibbons et al., 2024; Pasquini et al., 2025) and studies on positive and negative emotion-like states via cognitive biases (Bateson et al., 2011; Solvi et al., 2016). Indeed, the latter were also supported by findings of the authors of the critique (Procenko et al., 2024). Taken together, this accumulating evidence supports a precautionary approach to insect welfare as both scientifically reasonable and ethically responsible.

Author Contributions

Matilda Gibbons: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elisabetta Versace:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrew Crump:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bartosz Baran:** Writing – review & editing, Methodology, Conceptualization. **Lars Chittka:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Data Availability

The code and data are available on Figshare: https://figshare.com/s/a61515de60f5ed0da4c2.

Declaration of Interest

The authors declare no conflicts of interest.

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