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Associative learning: Unmet criterion for plant sentience

Commentary on [Segundo-Ortin & Calvo](#) on *Plant Sentience*

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Abstract: In a thought-provoking target article, Segundo-Ortin & Calvo (S&C) discuss the possibility that plants are sentient, focusing on a series of capacities normally attributed only to human and nonhuman animals. S&C propose learning as a marker for sentience. We review studies reporting associative learning in plants and find that they either lack essential controls or fail to produce replicable results. The capacity to learn has not yet been demonstrated in plants, so it cannot be used to support the hypothesis that plants are sentient. Further studies are needed. But agnosticism about sentience should not deter us from investigating unexpected new capacities in plants.

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Sentience refers to the ability to feel (Browning & Birch, 2022). This includes sensory experiences such as smelling a rose or the feeling of joy and happiness (Segundo-Ortin & Calvo, 2023). These are subjective states of the organism who feels them; they do not necessarily imply metacognitive skills (the ability to reflect upon a felt state) or empathy (feeling others' feelings) (Crump et al. 2022a). It is extremely difficult to determine whether and to what extent organisms are sentient because it is not possible to observe private inner experiences in any species. A possible way to infer whether and what an organism feels is to rely on indirect markers, such as

behavioral and cognitive responses to stimuli (or the organism's own verbal report, if it can speak) (Birch et al., 2020). In recent years, behavioral as well as conceptual analyses have focused on the possibility that decapod crustaceans and arthropods (a list which is likely to be extended in the future) may be sentient (Barron & Klein, 2016; Elwood, 2021, 2022; Fossat et al. 2014; Gibbons et al. 2022; Gibbons et al. 2022; Mikhalevich & Powell, 2020; Perry & Baciadonna, 2017; Solvi et al. 2016). This question has now been extended to plants by Segundo-Ortin & Calvo (2023) (S&C). In their stimulating target article, S&C analyze findings on several cognitive capacities commonly studied in human and nonhuman animals. They report that these capacities are also present in plants, either fully or partially (Calvo et al. 2020; Calvo & Trewavas, 2021; Garzón, 2007; Segundo-Ortin & Calvo, 2022).

Crump et al. (2022a,b) have proposed a useful set of criteria to test whether an animal is sentient. [See also Birch's 2023 commentary, this volume.] This has propelled discussion among scientists and policy makers about which taxa might be sentient in the hope that this might help in making ethical and moral decisions about how best to care for them. Crump et al.'s framework can also be used to test for the presence of sentience in plants. It is not possible to cover all of Crump et al.'s proposed criteria here, however, so we will consider only one of them that is also highlighted by S&C: *associative learning*. Crump et al. pay particular attention to *aversive* associative learning, in which animals learn to predict noxious stimuli from neutral stimuli through Pavlovian conditioning (see below) or ways they learn to avoid noxious stimuli through operant learning by reinforcement. Crump et al. explicitly exclude habituation and sensitization as insufficient to meet this criterion.

In their extensive review of the cognitive capacities of plants, S&C present evidence for different forms of learning, starting with habituation. We will follow Crump et al.'s agenda, setting aside habituation to concentrate on associative learning (although Colwill et al. [2023] have suggested that habituation may itself be associative). Besides, S&C's main example, the habituation of leaf-folding in *Mimosa pudica* (Gagliano et al. 2014), has been called into question because it lacked the dishabituation procedure required to demonstrate real habituation and because some of the leaf-folding data can be explained by motor fatigue (Biegler, 2018).

Associative learning is an adaptive change in behavior. It arises from acquiring information about contingencies between events or entities in the environment. The learning can take the form of modifying existing behaviors, or giving rise to new behaviors; and it is interpretable as reflecting either conscious or unconscious detection of the contingency (Rescorla & Holland, 1982). Several studies have addressed the question of whether plants are capable of associative learning, in particular, through Pavlovian learning (Haney 1969 cited in Applewhite, 1975; Armus, 1970; Holmes & Gruenberg, 1965; Holmes & Yost, 1966; Levy et al. 1970). This form of learning creates a link between an originally neutral conditional stimulus (**CS**) that anticipates a biologically relevant unconditional stimulus (**US**), resulting in the learning of a new conditional response (**CR**) to the **CS** (Pavlov, 1927).

The studies on Pavlovian learning in plants have again used *M. pudica*, but they remain inconclusive because they lack controls and have proved unreplicable (for detailed reviews please see Abramson & Chicas-Mosier, 2016; Adelman, 2018). There have also been recurrent problems with using stimulus durations similar to those used in animal learning studies (a few seconds), which may be too fast to be sensed effectively by plants. The leaf-folding reflex of

Mimosa is also atypical of the timescale at which plants usually respond. Hence results from this species may not be generalizable to other species (Adelman, 2018; Plebe 2023).

An example of nonreplicability cited by S&C as problematic is a study of the pea plant (*Pisum sativum*) (Gagliano et al. 2016). In one arm of a Y-maze, for three days, three times a day, seedlings of this plant were kept in the dark for 60 minutes and exposed to attractive blue light (henceforth 'L', the **US**). The plants grew towards the 'blue arm' by phototropism (unconditioned response, **UR**). To establish a Pavlovian association, air flow from a fan (henceforth 'F', a neutral **CS**) was delivered for 90 minutes in anticipation of the 60-min blue light exposure. There was an overlap of 30 minutes to better accommodate the plant's sensory and physiological scales (Adelman, 2018).

One group was conditioned with both F and L in the same arm of the maze [F + L], and the other group with F and L in opposite arms of the maze [F vs. L]. After training, the *experimental* groups of seedlings had three sessions with the **CS** (F) and the *control* groups had no stimulus at all. In the control groups, all the seedlings grew toward the arm in which the light had been presented in the last training session. In the two experimental groups ([F + L] and [F vs. L]), most of the seedlings grew toward the arm in which L was predicted by F (62% and 69%, respectively). The remaining seedlings grew toward the arm in which the light was presented during their last training session (38% and 31%, respectively). These results suggested that plant seedlings oriented toward the arm in which blue light was predicted by the fan, thus showing the capacity for associative learning. Unfortunately, further attempts to replicate this finding have so far failed, including one by the experimenters who had performed the original study (Gagliano et al. 2020; Markel, 2020a,b).

Another study mentioned by S&C is more interesting in light of Crump et al's emphasis on a learned response to a noxious stimulus (discussed earlier). This study (Bhandawat et al., 2020) focused on learning music (a mixture of classic music with natural sounds such as those produced by birds, insects and water) as a predictor of heat stress (37° temperature) in seedlings of *Arabidopsis thaliana*. Music and heat stress were presented twice a day (morning and evening) for 5 days. Conditioning consisted of 40min of music and 40min of heat, with an overlap of 20min and an interstimulus interval of 20 min. Three control groups were studied in parallel: one was exposed to music alone for 40min, one to heat stress alone for 40min and one was exposed to neither. All four groups were tested on day 6 with music alone and variation in heat-responsive genes was measured without any attempt to record changes in responses of the entire plant. Experience-dependent variation in heat-responsive gene levels was observed in various treatments, not only in the group that received the explicit pairing of music and heat stress. Thus, even setting aside the question of how plants would sense the sophisticated music provided and the more important criticism of the absence of an unpaired control group, which is necessary to demonstrate associative learning, the results of this experiment do not support associative learning in *Arabidopsis*.

Finally, S&C provide as further evidence for associative learning in plants a study suggesting that individual plants of the star-shaped flowering *Nasa poissoniana* (Loasaceae) adjust the timing of their pollen presentation to the actual frequency of pollination visits they experience (Mittelbach et al. 2019) [see their commentary, Henning & Mittelbach (2023)]. In these experiments, flowers were mechanically stimulated 3 times to simulate a pollinator visit,

with an interval of either 15min or 45min. On the next day, they were stimulated once and the movements of anthers aimed at presenting pollen were then observed for 50min to determine whether their prior experience had conditioned the timing of these movements. The experiment was repeated on four different days. Unfortunately, no control with unpredictable stimulation (i.e., 3 stimulations with a random intertrial interval) was performed. This would have been necessary to demonstrate the learning of a temporal contingency. Flowers stimulated for an interval of 15min tended to move anthers in anticipation of an expected visitor before the expected time of visitor recurrence; an equivalent pattern, however, was not statistically significant for flowers stimulated with an interval of 45min, thus casting doubt on the conclusion that plants learn to anticipate pollinator visits in an interval-specific way.

The present commentary thus explains why several authors are skeptical about the existence of true associative learning in plants (Adams, 2018; Cvrcková et al. 2009; Firn, 2004; Loy et al. 2021). Existing studies on plant associative learning face several major problems, some of which are: inappropriate experimental designs, lack of replicability, lack of proper control groups, high variability in the results reported, frequent use of small sample sizes (< 15 subjects per group) and inadequate knowledge about what a **CS** and a **US** could be for plants (Abramson & Chicas-Mosier, 2016; Adelman, 2018). Although some of these issues could be correctly tested experimentally, the general picture emerging from the studies to date support neither the inference that plants are capable of simple associative learning nor that they exhibit higher order cognitive processing (e.g., numerosity) and sentience.

The lack of consistent, reproducible evidence of learning and other cognitive capacities in plants is associated with recurrent debates on the necessity of a nervous system to mediate cognitive behaviors (Taiz et al. 2019). S&C argue that although plants have neither brains nor neurons to support cognition, the discovery of cellular signaling mechanisms that appear analogous to those of some animal neurons can be interpreted as partial support for cognition and sentience in plants. We find this doubtful. Ad hoc analogies and interpretations risk confirming whatever the proponent wants to see. Plants have signaling mechanisms at local scales (e.g., plant action potentials) and organismic scales (e.g., circulatory processes such as phloem) (Stahlberg, 2006), which both allow responding to environmental cues, damage, and other sources of information. However, these mechanisms do not provide an organism with the capacity to learn, encode, store, and retrieve information from memory, let alone capacities such as attention and sentience. Plants do exhibit goal-directed behavior in their reactions to environmental cues as well as in the signaling mechanisms described by S&C under “Plant Neurobiology,” but it is not clear why these responses should require sentience. All living organisms have the capacity to process and respond adaptively to environmental stimuli, yet this capacity is mostly completely unconscious (Ginsburg & Jablonka, 2021).

Two additional points need to be stressed: (1) simple associative learning, whether Pavlovian or operant, does not necessarily entail sentience because they can also occur without it (Shanks & St John, 1994) and can even be demonstrated in isolated neural circuits (e.g spinal cord preparations). This is why among Crump et al.’s criteria for sentience, associative learning is listed as one of several criteria that need to be met *jointly*. (2) In many living organisms, epigenetic mechanisms such as DNA methylation or histone modifications mediate various forms of memory (e.g., acetylation, deacetylation). These are responsible for dynamic changes in gene

transcription underlying memory formation and storage (Day & Sweatt, 2011; Marshall & Bredy, 2016). Epigenetic effects have also been interpreted as evidence that plants acquire “memories” of environmental experiences (stressful ones in particular) that enable them to adapt to environmental events (He & Li, 2018; Iwasaki & Paszkowski, 2014). Yet, such memories have never been demonstrated in the context of associative learning and cannot be considered a proof of sentience in plants.

We conclude that associative learning is still undemonstrated in plants. Far from discouraging investigations in this direction, however, we welcome Segundo-Ortin & Calvo’s current and future efforts. Properly designed and replicable experiments are needed. In the meantime, agnosticism, too, is needed, before we begin to attribute sentience to plants and speculate about its ethical implications.

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