

# Experience teaches plants to learn faster and forget slower in environments where it matters

Monica Gagliano · Michael Renton ·  
Martial Depczynski · Stefano Mancuso

Received: 6 September 2013 / Accepted: 20 December 2013 / Published online: 5 January 2014  
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**Abstract** The nervous system of animals serves the acquisition, memorization and recollection of information. Like animals, plants also acquire a huge amount of information from their environment, yet their capacity to memorize and organize learned behavioral responses has not been demonstrated. In *Mimosa pudica*—the sensitive plant—the defensive leaf-folding behaviour in response to repeated physical disturbance exhibits clear habituation, suggesting some elementary form of learning. Applying the theory and the analytical methods usually employed in animal learning research, we show that leaf-folding habituation is more pronounced and persistent for plants growing in energetically costly environments. Astonishingly, *Mimosa* can display the learned response even when left undisturbed in a more favourable environment for a month. This relatively

long-lasting learned behavioural change as a result of previous experience matches the persistence of habituation effects observed in many animals.

**Keywords** Behaviour · Ecological trade-offs · Information · Anti-predator responses · Learning · Memory

## Introduction

By definition, learning is a process for acquiring memories by which adaptive changes in an organism's behaviour arise as a result of experience (Okano et al. 2000). Because learning is not directly observable (i.e. we cannot directly access the traces past experiences have left in the memory of an individual), it can only be detected operationally when an observed behaviour has changed due to a past experience that the individual itself remembers (Kawecki 2010). Recognizing and quantifying such behavioral changes has proven to be no easy task in human and animal subjects, let alone in plants whose ability to *behave* is still an underappreciated phenomenon (Karban 2008). Even so, researchers have attempted to experimentally test (Applewhite 1972; Thellier et al. 1982, 2000) as well as conceptually validate [through mathematical models (Demongeot et al. 2000; Bose and Karmakar 2008; Inoue 2008)] the idea that plants can learn and remember, since Sir J. C. Bose (1858–1937) first proposed this over a century ago. What these studies have shown is that plants are indeed capable of memory function (see Trewavas 2003; Thellier and Lüttge 2013); in all cases, the behaviour of the different plants included an ability to store and recall biological information in order to produce a specific (and somewhat predictable) developmental (Karban and Niiho 1995; Goodrich and Tweedie 2002; Reyes et al. 2002), physiological (Baldwin and

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Communicated by Richard Karban.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-013-2873-7) contains supplementary material, which is available to authorized users.

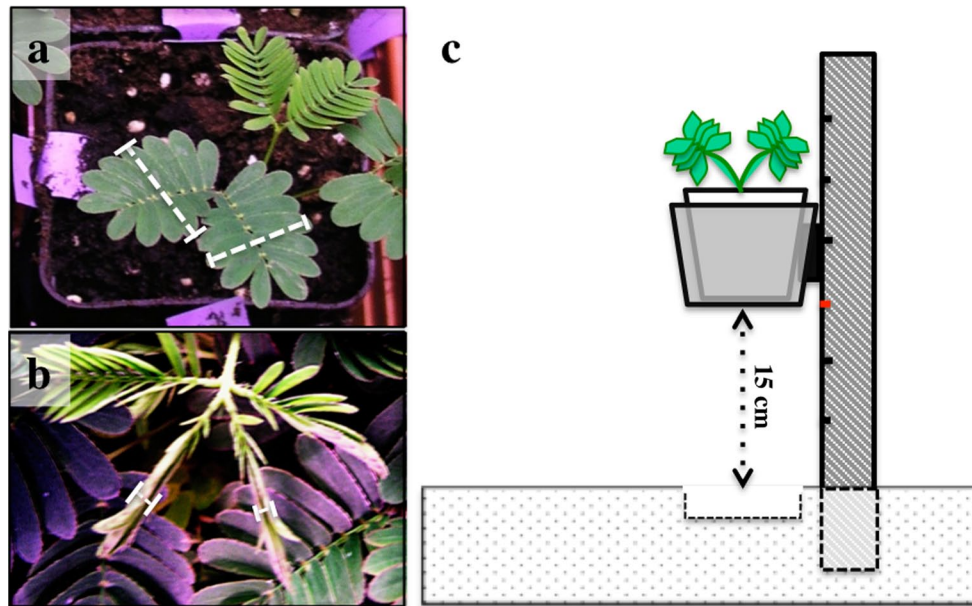
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M. Gagliano (✉)  
Centre for Evolutionary Biology, School of Animal Biology,  
University of Western Australia, Crawley, WA 6009, Australia  
e-mail: monica.gagliano@uwa.edu.au

M. Renton  
School of Plant Biology, University of Western Australia,  
Crawley, WA 6009, Australia

M. Depczynski  
AIMS, The Oceans Institute, University of Western Australia,  
Crawley, WA 6009, Australia

S. Mancuso  
LINV, Department of Plant, Soil and Environmental Science,  
University of Firenze, Sesto F.no (FI), Firenze, Italy



**Fig. 1** Controlled drop system for habituation training of *Mimosa* plants. The sensitive plant *Mimosa pudica* was chosen as the ideal model for this study, because of its capacity to rapidly fold its leaves in response to physical disturbance. Disturbance causes the subleaflets to rapidly fold up along the stem of each leaflet, and even the leaflet and leaf stem to droop downwards in a matter of seconds. Recovery time is variable and leaves may take a few seconds to several minutes to re-open fully. The maximum leaf breadth before and after training was carefully measured tip-to-tip (as indicated by the

white dotted line; **a** and **b**). The set-up consisted of a plastic vessel mounted with variable hangers onto a marked steel rail, which was in turn secured to a foam base. Tightly fitted in the host vessel, individual potted plants were manually elevated to the 15-cm height mark and allowed to drop by sliding along the rail. The shallow depression in the foam base at the landing point of the vessel prevented it from bouncing at impact. The set-up ensured that a standard level of disturbance was administered to all plants and it was sufficient to force the closure of all leaves (**c**)

Schmelz 1996; Ruuhola et al. 2007), morphological (Dostál 1967; Thellier et al. 1982, 2000) or epigenetic response (Sung and Amasino 2004; Molinier et al. 2006; Kinoshita and Jacobsen 2012 and related articles in special focus issue). Defined by a fixed ‘blueprint’, such induced adaptive responses are extremely valuable at adapting the organism to its environment and, in fact, are part of an innate behavioral repertoire of an organism. However, this leaves open the question of whether plants, like animals, can show learned behavioral responses (i.e. a behaviour that an individual develops by being taught).

It appears that the major impediment to resolving this issue lies in the absence of a robust behavioral test purposely designed for recognizing the presence of functional features typical of systems capable of true memory and learning (Cvrčková et al. 2009). We have performed this test here by applying ecological theory developed for animals (Lima 1998) to the defensive leaf-folding reflex of *Mimosa pudica* (hereafter referred to as ‘*Mimosa*’; Fig. 1a, b), a plant known for its leaf-folding behaviour in response to physical disturbance (Applewhite 1972), to examine the behavioral phenomenon of habituation in this plant. Often considered to be the simplest form of learning, habituation is an adaptive process that enables an organism to focus on

the important information in its environment, while filtering out stimuli or events that, over time, have repeatedly proven to be irrelevant and innocuous (Thorpe 1963; Eisenstein et al. 2001; Hemmi and Merkle 2009; Rankin et al. 2009). Hence to meet these criteria, a plant must be able to select relevant things to memorize and actively access those memories to modify the timing, quality or quantity of its behavioral response when exposed to external stimuli. In this study, we conducted a series of experiments to test whether *Mimosa* can truly learn under these criteria, how long would it remember what was learned, and to what extent the environment influences its learned behaviour.

The rapid closure of *Mimosa*’s delicate leaves in response to mechanical disturbance is believed to be a defence tactic to reduce predation risk (Eisner 1981; Braam 2005; Fromm and Lautner 2007) and this leaf-folding behaviour may be adaptive (Cahill et al. 2013). Moreover, leaf closure is known to be sensitive to light levels and specifically, *Mimosa* plants are more likely to risk predation when foraging at lower light intensities (Jensen et al. 2011). In this study, we subjected plants to repeated treatments that initially caused leaves to close but the response to which was unnecessary and non-adaptive, to test whether plants could learn that these stimuli should

be ignored. Specifically in our first set of experiments, we examined whether *Mimosa* plants grown under low- (LL) and high-light (HL) conditions differ in their rate of learning about an environmental stimulus and retaining what is learned. Based on the predictions by Jensen et al. (2011) that predation risk (i.e. showing visible leaves) should be traded off with energetic gain (i.e. opportunity for photosynthesis with open leaves), we expected that individuals in the low-light environment would be the faster learners and more likely to retain the learned behaviour for longer. In a second set of experiments, we manipulated the light conditions individuals experienced over time, thereby changing the trade-off between predation risk and energetic reward. We expected that individuals originally grown under the HL environment and later experiencing LL conditions would become faster learners, while those grown under the LL environment and transferred to higher light conditions would display a reduced response.

## Materials and methods

### Experimental set-up and design

All experiments were done in a 5.30-m<sup>2</sup> growth room with three compartments, separated from each other by sheets of black plastic. All training trials were conducted in the middle compartment, whereas all experimental plants were held in one of the side compartments, fitted with fluorescent lights to produce LL (90  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and HL (230  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) conditions respectively. Light intensity was measured directly above the plants in each compartment prior to the commencement of the experiments. In a first series of experiments, we tested whether light conditions influenced how *Mimosa*'s leaf-folding reflex habituates and how long habituation persists (i.e. short-term; after plants were left undisturbed for 6 days). In the second series of experiments, we examined long-term habituation of the reflex by re-testing 26 previously trained plants that were left undisturbed for 1 month and assessing whether *Mimosa*'s habituated response, like in animals (Sztarker and Tomsic 2011), can be evoked only if the plant is tested in the same environment where it was trained. All similarly sized (6–8 cm tall) plants were obtained from a local commercial nursery (Cooperative Legnaia, Firenze) and directly transferred to the experimental growth room at the Department of Plant, Soil and Environmental Science at the University of Firenze. Here, plants were grown individually in 10-cm round plastic pots with a standard mixture (1:1, volume/volume) of loamy soil and organic compost (Neuhaus N2). For the entire study, we maintained the health and growth of all plants by fertilizing with 1/5-strength Hoagland's solution (Hoagland and Arnon 1950) and

watering as needed (i.e. plants did not express any visible signs of nutrient or water stress). Throughout the study, all plants were exposed to identical 12-h light:12-h dark cycle conditions, 60–70 % ambient humidity and 21–24 °C temperature profiles (air temperature in the compartments housing the plants was recorded using an EasyLog EL-USB 2 data-logger).

### Habituation training of *Mimosa*'s leaf-folding reflex

Consistent with the hypothesis of a trade-off between predation risk and energetic demand, we examined the effects of HL and LL conditions on the habituation training of the defensive reflex of this plant. Plants in individual pots were randomly assigned to either HL or LL conditions and then left undisturbed for 5 days until their scheduled training day. Plants were trained using a custom-designed controlled drop system (Fig. 1c) for administering a standardised stimulus (i.e. a 15-cm fall or drop) that successfully elicits the leaf-folding reflex. The response of individual plants was estimated by averaging the response of three randomly selected leaves and measured by how much these leaves re-opened (if at all) after a train of drops. Specifically, each leaf was carefully measured tip-to-tip using digital callipers; its response was then quantified as the maximum leaf breadth (mm) measured immediately at the end of a train of drops (Fig. 1b) relative to the undisturbed pre-stimulus maximum breadth (Fig. 1a). Both before and after a train, the three leaves from each plant were measured in quick succession by the same individual observer.

We first tested whether *Mimosa* is able to remember a stimulus based on a brief, one-off experience by administering one drop only in the morning and then again 8 h later to a group of 16 naïve plants ( $n = 8$  per light treatment). Next, we trained a group of 56 naïve plants ( $n = 28$  per light treatment) by administering seven consecutive trains of 60 drops each at either 5- or 10-s inter-stimulus intervals, delivered at increasingly longer inter-train intervals but all within a single day.

### Dishabituation test

To test whether the trained response in *Mimosa* was due to learning rather than caused by other processes such as fatigue or sensory adaptation (see Rankin et al. 2009), we conducted a standard dishabituation test by assessing the plants' response following the presentation of a novel stimulus. Dishabituation occurs when a novel stimulus [generally, more intense within a given stimulus modality (Rankin et al. 2009; Kenzer et al. 2013)] is briefly presented and elicits the recovery of the original behavioural response (in *Mimosa*'s case, leaf closure). The recovered response does not necessarily have to return to the initial levels observed

before habituation (Wiel and Weeks 1996), but may be less than or equal to baseline levels (Thompson 2009) or those seen immediately prior to the presentation of the dishabituation stimulus (Petrinovich and Widaman 1984).

In this study, we tested for dishabituation by placing individual plants in a close-fitting foam container attached to a shaker plate (Heidolph, Titramax 1000) and shaking them at 250 r.p.m. for 5 s (Dis-hab treatment). This stimulus was chosen on the basis that it did not differ significantly in modality from the habituating stimulus (Grissom and Bhatnagar 2009), but was nevertheless novel to the plant, and hence able to elicit the rapid leaf-folding reflex as effectively as the brief, one-off experience of the habituating stimulus when delivered during the initial test described above.

#### Short-term memory of the habituated leaf-folding response

To examine the short-term effects of 1-day training on the retention of the habituated response, all trained plants from both light treatments were re-tested 6 days later by observing the leaf-folding response as they received one train of 60 drops.

#### Long-term memory in *Mimosa* exposed to a new environment

To test the effects of a changing environment, we conducted a second series of experiments where 13 plants (including five control plants) previously habituated to LL conditions were transferred to the HL treatment and vice versa, and then re-tested 28 days later by undergoing the complete training (i.e. 7 consecutive trains of 60 drops delivered throughout 1 day).

#### Statistical design and analyses

Analyses were conducted in R using the core and lme4 packages (Bates et al. 2011) to fit and compare linear mixed-effects models, the appropriate modern technique for dealing with this kind of nested, repeated measures data. For each analysis, we first fitted a full model and then conducted step-wise simplification to determine which terms were significant in the standard way (Crawley 2007). Specifically, at each step a simplified model with one term dropped from the previous best model (starting with the full model) was compared to the previous best model using both Akaike's information criterion (AIC) and a likelihood ratio  $\chi^2$ -test (see Electronic Supplementary Material for more details). AIC values were computed for each of the candidate models and the model with the lowest AIC value was selected in the standard way as the best model of the observed data (Burnham and Anderson 2002).

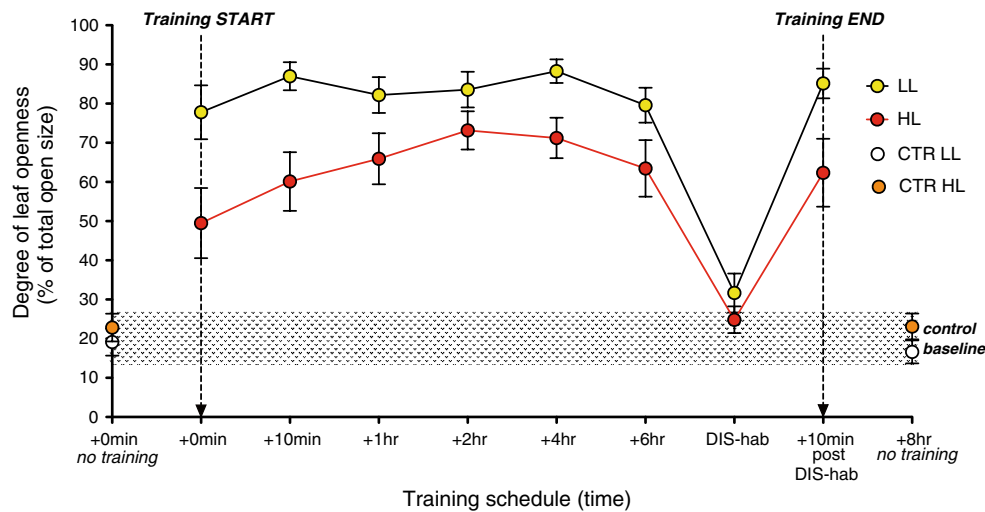
## Results

### Habituation training of *Mimosa*

The initial test consisting of a brief, single-drop experience of the standardised stimulus (Fig. 1c; see details in “Materials and methods”) confirmed that irrespective of the light treatment, such a short-lived experience offers insufficient opportunities for this plant to learn (repeated-measures ANOVA,  $P = 0.24$ ,  $F_{1,14} = 1.49$ ). We used these untrained responses to construct light-specific control baselines (Fig. 2). Instead, we found that the leaf-folding reflex of plants undergoing full training (i.e. seven consecutive trains of 60 drops in a single day) habituates rapidly (Fig. 2). We observed leaves starting to re-open even before the first train of drops was delivered in full (i.e. after the first four to six drops) and when repeatedly elicited over the course of the training, leaves were not only completely open by the end of a train but also stopped closing altogether. Moreover, the leaf-folding reflex habituated more rapidly and leaves re-opened more fully under LL (Fig. 2; difference between light profiles, linear mixed-effects model,  $P < 0.0001$ ,  $\chi^2 = 60$ ). This result is perhaps not surprising from an energetic point of view given that *Mimosa*'s photosynthetic rate drops by up to 40 % when its leaves are closed (Hoddinott 1997), although it is important to note that plants did not exhibit any signs of light stress (e.g. no observable differences in size and appearance). The result shows that a greater ability to ignore a recurrent, yet harmless stimulus, in order to minimize energy waste and optimize opportunities to forage for light is to be expected in environments where the available energy is more limited.

### Response of the habituated leaf-folding reflex to a novel stimulus

We found that the habituated response returned to original baseline levels when the novel stimulus—a shake at 250 r.p.m. for 5 s—was applied (i.e. dishabituation; Fig. 2; differences between +6 h and DIS-hab,  $P < 0.0001$ ,  $\chi^2 = 350$  for both environments). This is an important result because it rules out the alternative explanation for a decrease in re-opening response due to exhaustion of energy or other resources. Moreover, it was interesting to find that the habituated response is only provisionally overridden [as observed in animal studies (Grissom and Bhatnagar 2009)] and instead can be fully elicited again when the original familiar drop stimulus is re-presented 10 min later (Fig. 2; differences between DIS-hab and +10 min post-DIS-hab,  $P < 0.0001$ ,  $\chi^2 = 401$ , and no difference between +6 h and +10 min post-DIS-hab in both environments,  $P = 0.19$ ,  $\chi^2 = 1.7$ ). The maintenance of the habituated response despite dishabituation confirms that the response



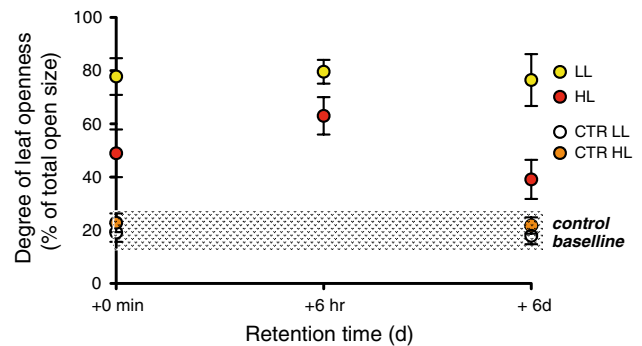
**Fig. 2** Habituation training profiles in high- (HL) and low-light (LL) environments. In a single training day, each plant received the first train of 60 drops at time zero (+0 min) and the second train 10 min later, a sufficient inter-train interval to allow all plants to spontaneously recover and fully re-open their leaves. Subsequent trains were then delivered 1, 2, 4 and 6 h later. Immediately after the last train of drops was delivered (at +6 h), individual plants were shaken at 250 r.p.m. for 5 s (DIS-hab). Following spontaneous recovery, plants were administered a final train of drops (+10 min post-DIS-hab). Throughout this experiment, a total of 20 naïve plants (CTR;  $n = 10$

per light environment) were also tested by delivering one drop only in the morning of training (i.e. control baseline indicated by the dotted grey area). There were differences between inter-stimulus intervals (ISI) levels for the LL treatment ( $P < 0.0001$ ,  $\chi^2 = 21$ ), but not for the HL treatment ( $P = 0.5$ ,  $\chi^2 = 1.2$ ); for clarity this plot shows data for both ISI levels pooled. The maximum leaf breadth at the end of a train of drops is expressed as a percentage of the maximum breadth prior to the delivery of a train (i.e. degree of leaf openness) and shown as mean  $\pm$  95 % confidence interval (CI)

decrease cannot be attributed to fatigue or sensory adaptation; instead it denotes the selective nature of habituation, an active learning process whereby plants (like animals) perceive an innocuous stimulus but choose not to respond to it while still remaining responsive to the surrounding environment.

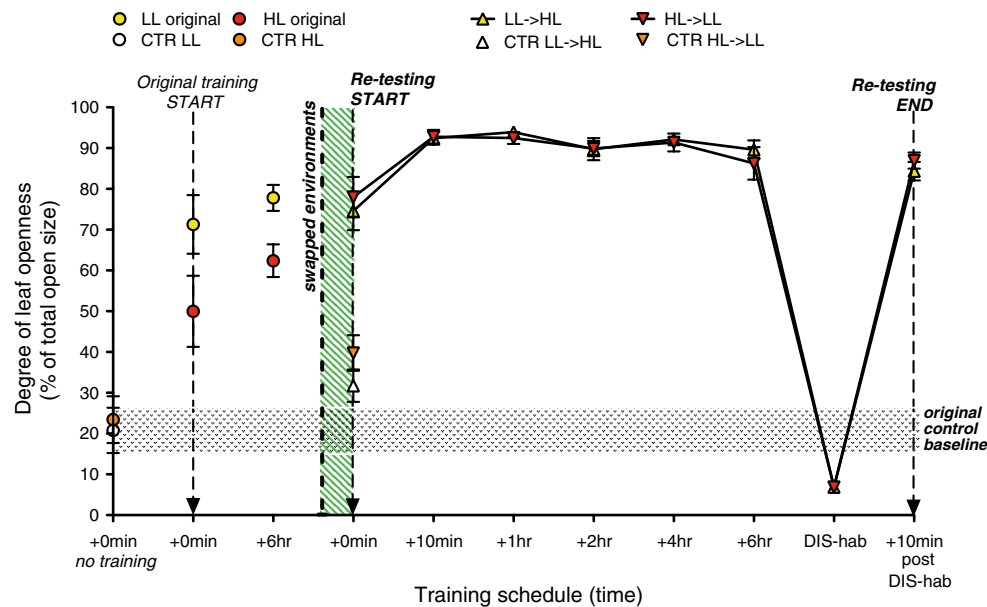
Short-term memory of the habituated leaf-folding response

In our first experiment, we examined the effects of 1-day habituation training on the retention of the habituated response to find that changes in behaviour persist virtually unchanged 6 days later under LL conditions (Fig. 3; no difference between +6 h and +6d,  $P = 0.7$ ,  $\chi^2 = 0.137$ ), but declined by a considerable 23 % under more favourable HL conditions (Fig. 3; difference between +6 h and +6d,  $P < 0.0001$ ,  $\chi^2 = 23.4$ ). Because we now know that *Mimosa* plants can modify their leaf-folding behaviour rapidly (i.e. re-opening after the first four to six drops during initial training) and we quantified the degree of leaf openness only at the end of the full train of drops, we recognise that we cannot irrefutably exclude the alternative hypothesis that what we observed at the end of the full train of drops (as shown in Fig. 3) is simply rapid re-habituation rather than short-term memory. It is important to note, however, that during the delivery of the train of drops on day 6, we observed that some individuals did not close their leaves



**Fig. 3** Short-term effects of 1-day habituation training. Two groups of 28 trained plants ( $n = 14$  per light treatment;  $n = 7$  per ISI group) were re-tested 6 days later, respectively. Habituation of the leaf-folding reflex was partially recovered in HL plants, but fully retained in LL ones. During the entire period of the experiment, a total of 20 naïve plants (CTR;  $n = 10$  per light environment) was also tested by delivering one drop only in the morning of training (i.e. control baseline indicated by the dotted grey area). Percentage re-opening of the maximum leaf breadth (i.e. degree of leaf openness) is shown as mean  $\pm$  95 % CI. For abbreviations, see Fig. 2

fully when dropped, whilst those that did, reopened them following just two to three drops. Such shortened response times suggests that these plants have a memory of previous experience. Moreover, our results suggest that plants in an energetically favourable environment can afford to reverse a formerly habituated response, and such reversal



**Fig. 4** Long-term retention of the habituated leaf-folding reflex. The habituated response of LL plants first re-tested after being held undisturbed for 28 days (indicated by the green shading) under the new HL conditions (i.e. +0 min after swap) could be evoked unchanged. However when first re-tested in the new LL environment, the response of HL plants was significantly increased. After the swap,

differences between the two light treatments over time disappeared. Throughout this swap experiment, a total of 12 control plants ( $n = 6$  CTR LL  $\rightarrow$  HL;  $n = 6$  CTR HL  $\rightarrow$  LL) were also re-tested by delivering one drop only in the morning of training. Percentage re-opening of the maximum leaf breadth (i.e. degree of leaf openness) is shown as mean  $\pm$  SE. For abbreviations, see Fig. 2

can occur within a relatively short time-frame. Contrarily, plants experiencing an energetically more demanding environment retained the habituated response unchanged to minimize energy loss, suggesting that stable habituation of *Mimosa*'s leaf-folding reflex can be produced and depends on the environmental context.

#### Long-term memory in *Mimosa*

Strikingly, the habituated response of LL plants first re-tested after being held undisturbed for 28 days (indicated by the green shading in Fig. 4) under the new HL conditions (i.e. +0 min after swap) remained unchanged ( $P = 0.51$ ,  $\chi^2 = 0.44$  compared to +0 min before swap;  $P = 0.16$ ,  $\chi^2 = 1.96$  compared to +6 h before swap). However, when first re-tested in the new LL environment, the response of HL plants was significantly increased ( $P < 0.0001$ ,  $\chi^2 = 26$  compared to +6 h before swap;  $P < 0.0001$ ,  $\chi^2 = 30$  compared to +0 min before swap). After the swap, there was no difference between the two light treatments over time ( $P = 0.9$ ,  $\chi^2 = 3.5$ ; Fig. 4). It is interesting to note that the results from the HL plants transferred to the LL treatment indicate that plants quickly adapt their learned behavioural responses to new environments, especially when the available energy has become more limited (i.e. exhibit an enhanced ability to ignore the recurrent yet harmless stimulus than they had done before). Even more interesting,

however, are the results from the LL plants transferred to the HL treatment. Contrary to our expectation of a reduced response, these plants continue to fully exhibit the learned behaviour that, in fact, proves to be especially useful in the new environment because it now affords maximum foraging opportunity. Overall, plants whose leaf-folding reflex had habituated through earlier training in one light treatment still exhibited the learned behaviour in the new light condition almost a month later (Fig. 4), demonstrating that a change in the training environment does not prevent this plant from expressing long-term habituation. Moreover following training 28 days later, plant responses in both treatments are more pronounced (i.e. greater degree of leaf openness) and overall variance levels in such responses are remarkably reduced by 85.3 % for LL  $\rightarrow$  HL and 63.1 % for HL  $\rightarrow$  LL plants when compared to the same responses during initial training (Fig. 4).

This not only excludes the possibility that this is simply rapid re-habituation, it demonstrates an increase in the tendency of individuals to take a specific action in response to a known stimulus based on prior learning.

#### Discussion

Most probably the oldest form of learning, habituation can be observed across a wide range of organisms. Clearly

being able to generalize a learned response is of considerable adaptive value, as long as the response is stimulus-specific enough to allow the organism to keep reacting to unknown, potentially dangerous, stimuli (Rankin et al. 2009). Our results indicate that this is the case for *Mimosa*; in fact, all experimental plants irrespective of their light treatment respond promptly by closing their leaves when a novel dishabituating stimulus is presented (Fig. 4), hence exhibiting stimulus specificity. Concomitantly, *Mimosa* can acquire an enduring memory of a past event, whereby the plant recognizes and generalizes the learned stimulus even when the context in which the memory was generated has changed (considering that each plant was immediately returned to its specific light environment in between each training session, the ‘context’ a plant experienced during a whole training day would be best described by the combined ‘holding’ and ‘testing’ environment). In other words, *Mimosa*’s proficiency in responding appropriately to a known stimulus within a new context does not require brand-new learning but the capacity to ‘remember’ (i.e. recognize that a present cue has been previously encountered) and adjust slightly. It seems, then, that the trade-off between stimulus generalization and stimulus specificity is an essential feature of how plants (akin to animals) respond to their environment. As recently pointed out in the animal literature (Sztarker and Tomsic 2011), the final decision of how an organism will behave is based on the similarity between the known stimulus and the new one, assessed along a perceptual gradient that is relevant to the individual species.

In this study, we have demonstrated the acquisition and expression of a long-lasting memory for a learned behaviour in this plant, but we have left unanswered the fundamental question of the mechanisms underlying this deceptively simple form of learning, the biological basis of which we still do not fully understand in any organism [but see work on invertebrates, such as crayfish (Krasne and Teshiba 1995); sea slugs (Glanzman 2009); and nematodes (Giles and Rankin 2009)]. The current hypothesis for neural organisms (primarily simpler invertebrate animals) proposes that there are a number of cellular mechanisms that are differentially activated to mediate short- and long-term habituation (Rankin et al. 2009) and specifically, the long-term effects involve voltage-gated ion channels, neurotransmitters and changes in protein synthesis [in *Aplysia californica* (Esdin et al. 2010)]. Interestingly, the physiological and cellular mechanisms of leaf movements associated with fast responses to environmental stimuli in plants like *Mimosa* have been known for some time (reviewed by Moran 2007), revealing that these plants employ sensory and motor pathways (Shepherd 2012) with the direct involvement of voltage-gated ion channels (Fleurat-Lessard et al. 1997), neurotransmitters (Roshchina 2001) and

changes in protein synthesis [e.g. aquaporin (Uehlein and Kaldenhoff 2008)], all of which may also be utilized during the learning process. Hence, we propose that plants such as *Mimosa* offer a unique opportunity for investigating the biology of learning and memory, notwithstanding the current belief that plant behaviour is a collection of simple, automata-like routines. After all, considerable progress in this research field over the last few decades has been made possible by the study of invertebrates as model species, a choice originally based on the assumption that these were simple animals performing simple tasks [yet, they have turned out to be far more complicated than we assumed (reviewed by Tomsic et al. 2009)].

So how do plants learn and remember?

Plants may lack brains and neural tissues but they do possess a sophisticated Ca-signalling network in their cells (Yang and Poovaiah 2003) similar to those underlying memory processes in animals (Perisse et al. 2009). Specifically, intracellular Ca ( $[Ca^{2+}]_i$ ) signals are known to regulate a large variety of functions in all biological organisms (Berridge et al. 2000), including memory processing and formation of memory imprints of past events ranging from minutes to generations through gene expression (Perisse et al. 2009; Gális et al. 2009). Interestingly in animals, fluctuations in  $[Ca^{2+}]_i$  during learning seem to be essential in priming the organism for the formation of long-term memory, without affecting short-term memory (Perisse et al. 2009; Bauer et al. 2002). In plants, this same  $[Ca^{2+}]_i$  system is already known to contribute to the formation of stress imprints (Conrath et al. 2001) and may be responsible for the long-term memory we observed in *Mimosa*. As a matter of fact, in both animals and plants, fluctuations in  $[Ca^{2+}]_i$  levels are directly linked to stimulus–response coupling through changes in the concentration of small molecules and proteins, including calmodulin (CaM). What is really interesting about this  $Ca^{2+}$  sensor is that CaM is one of the most conserved  $Ca^{2+}$ -binding proteins in eukaryotes [ $>70$  % sequence alignment similarity between animals and plants, albeit plants have a much larger repertoire of genes coding for CaM-target proteins (Yang and Poovaiah 2003)]. The  $Ca^{2+}$ /CaM signalling system controls the expression of genes whether their transcription occurs in the neurons of a big-brained animal (Limback-Stokin et al. 2004) or in the root apex cells of a plant (Kim et al. 2009). This might be the starting point from which a minute, yet measurable  $Ca^{2+}$ /CaM signal acting on voltage-gated ion channels (Halling et al. 2005) and their production of electrical waves (Yellen 1998), culminates in the formation of memories and expression of the most remarkable behaviours in animals [e.g. *Aplysia* (Esdin et al. 2010)] and plants alike.

Beside the  $\text{Ca}^{2+}$  model, there is another possible hypothesis to explain memory mechanisms in plants at the cellular level. It is now known that many non-neural cells are capable of processing information via ion flows generated by ion channels/pumps and propagated by cell:cell junctions [e.g. cardiac tissue (Chakravarthy and Ghosh 1997); bone cells (Turner et al. 2002)]. Simulating neural network-like dynamics, stable bioelectrical gradients among non-excitable cells could store information, memories and exhibit many other properties usually reserved for brains (Tseng and Levin 2013). Plants are no exception, especially when we consider that they have, in fact, well-developed pathways for the effective transmission of information via electrical signals (Volkov et al. 2008).

Additionally at the molecular level, the role of epigenetic reprogramming has been increasingly identified as a promising candidate mechanism for learning and memory storage in plants (Thellier and Lüttge 2013) and more generally, across all taxa (Ginsburg and Jablonka 2009; Ledón-Rettig et al. 2013). In principle, behavioral responses can be modified or refined through the integration of environmental experiences (i.e. learning) via changes in chromatin status [modulated by acetylation and methylation of DNA and histone proteins (Allis et al. 2007)]. In fact, it is well documented that epigenetic variations can be induced by external stimuli (Boyko and Kovalchuk 2008; Alvarez et al. 2010; Yaish et al. 2011) and more specifically, that changes in chromatin methylation patterns are strongly produced following environmental stress (e.g. Molinier et al. 2006; Chinnusamy and Zhu 2009; Verhoeven et al. 2010; Ding et al. 2012) and retained as a source of ‘stress memory’ [e.g. vernalization, whereby the flowering of plants requires priming by previous exposure to chilling (Sung and Amasino 2004); induced plant defences, whereby an anti-herbivore or anti-pathogen response is primed by environmental cues that reliably indicate an increased probability of attacks prior to their occurrence (Conrath 2009); acquired tolerance to drought stress, whereby exposure of young plants to a mild salt treatment primes enhanced drought tolerance in adult plants (Han and Wagner 2013)]. Yet, it should be cautiously noted here that these stress-induced changes in plant response resulting from previous experiences involve a priming input that modifies chromatin patterns but does not lead to an immediate phenotypic response [i.e. memory with delayed output (Ginsburg and Jablonka 2009)]. These are quite different from habituated responses like the one shown by the *Mimosa* plants in this study, where an input to the gene may produce a behavioral output that operates as a negative regulator of the gene, imposing an inhibitory epigenetic marking which, in turn, leads to a smaller behavioral response when recurring stimulations of the same kind activate that gene [i.e. memory of inhibitory modifications and recall (Ginsburg and Jablonka 2009)]. Secondly,

changes in chromatin marks seem to always require severe stress conditions and prolonged exposure to such conditions (reviewed by Pecinka and Mittelsten Scheid 2012), opening an important question as to whether epigenetic variations mediate memory and learning processes when organisms are not under stress. Obviously, different circumstances and environmental conditions affect the behavioral actions an organism takes in different ways; in turn, different behaviours during a learning experience can lead to functionally significant differences in the information acquired as a result of the experience [i.e. a learned response develops within the lifetime of an individual based on sensory feedback (Kawecki 2010)]. While any environmental factor can become a stressor, stress per se [i.e. the organism fails to respond adequately to unfavourable conditions (Pecinka and Mittelsten Scheid 2012)] is induced only when the intensity of the conditions is too high or too low (i.e. beyond the relative sensitivity and threshold value of the organism). By creating situations in which individuals had an opportunity to acquire useful information without being exposed to such extreme conditions, our study suggests that it is worth investigating the epigenetic reprogramming more broadly to gain a complete view on its potential role as a likely molecular mechanism underlying learning and memory functions across living systems.

## Conclusion

Once the domain of psychologists, research on learning and memory has extended its reach beyond the study of human behaviour to include several animal species and more recently, even machines. The recent emergence of interdisciplinary approaches like cognitive ecology, which focuses on the functional roles of learning in nature, and how different species obtain and make use of information about their environment to survive (Dukas 2004), have revealed the exceptional importance that the ability to learn plays in biological evolution as a whole (Kawecki 2010). Because of the very fact that much of the advances in learning research come from humans and animals, the acquisition and use of information through the learning process is implicitly accepted to be contingent on neuronal processes (or artificial neural networks modelled on their biological counterparts, in the case of machines), a view that inevitably excludes non-neural organisms such as plants from the behavioral realm of learning, memory and decision-making (but see Trevas 2003). What we have shown here, however, leads to one clear, albeit quite different, conclusion: the process of remembering may not require the conventional neural networks and pathways of animals; brains and neurons are just one possible, undeniably sophisticated, solution, but they may not be a necessary requirement for learning.



**Acknowledgments** We thank Elisa Azzarello and Elisa Masi for assistance with setting up the light environments, and Leigh Simmons, Joseph Tomkins, Anthony Trewavas, Daniel Robert for valuable comments on the manuscript. This study was supported by Research Fellowships from the University of Western Australia and the Australian Research Council to M. G. and research funding from European Commission to S. M.

## References

- Allis CD, Jenuwein T, Reinberg D, Caparros ML (2007) Epigenetics. Cold Spring Harbor Laboratory Press, New York
- Alvarez ME, Nota F, Cambiagno DA (2010) Epigenetic control of plant immunity. *Mol Plant Pathol* 11:563–576
- Applewhite PB (1972) Behavioral plasticity in the sensitive plant, *Mimosa*. *Behav Biol* 7:47–53
- Baldwin IT, Schmelz EA (1996) Immunological “memory” in the induced accumulation of nicotine in wild tobacco. *Ecology* 77:236–246
- Bates D, Maechler M, Bolker B (2011) lme4: Linear mixed-effects models using Eigen and Eigen. R package version 0.999375-42. <http://CRAN.R-project.org/package=lme4>
- Bauer EP, Schafe GE, LeDoux JE (2002) NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *J Neurosci* 22:5239–5249
- Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signalling. *Nat Rev Mol Cell Bio* 1:11–21
- Bose I, Karmakar R (2008) Simple models of plant learning and memory. *Phys Script* T106:9–12
- Boyko A, Kovalchuk I (2008) Epigenetic control of plant stress response. *Environ Mol Mutagen* 49:61–72
- Braam J (2005) In touch: plant responses to mechanical stimuli. *New Phytol* 165:373–389
- Burnham KP, Anderson DR (2002) Model selection and multimodal inference: a practical information-theoretic approach. Springer, New York
- Cahill JF Jr, Bao T, Maloney M, Kolenosky C (2013) Mechanical leaf damage causes localized, but not systematic, changes in leaf movement behaviour of the sensitive plant, *Mimosa pudica*. *Botany* 91:43–47
- Chakravarthy SV, Ghosh J (1997) On Hebbian-like adaptation in heart muscle: a proposal for ‘cardiac memory’. *Biol Cybern* 76:207–215
- Chinnusamy V, Zhu JK (2009) Epigenetic regulation of stress responses in plants. *Curr Opin Plant Biol* 12:133–139
- Conrath U (2009) Priming of induced plant defense responses. *Adv Bot Res* 51:361–395
- Conrath U, Thulke O, Katz V, Schwindling S, Kohler A (2001) Priming as a mechanism in induced systemic resistance of plants. *Eur J Plant Pathol* 107:113–119
- Crawley MJ (2007) The R book. Wiley, Chichester
- Cvrčková F, Lipavská H, Žárský V (2009) Plant intelligence: why, why not or where? *Plant Signal Behav* 4:394–399
- Demongeot J, Thomas R, Thellier M (2000) A mathematical model for storage and recall functions in plants. *C R Acad Sci III* 323:93–97
- Ding Y, Fromm M, Avramova Z (2012) Multiple exposures to drought ‘train’ transcriptional responses in *Arabidopsis*. *Nat Commun* 3:740
- Dostál R (1967) On integration in plants. Harvard University Press, Cambridge
- Dukas R (2004) Evolutionary biology of animal cognition. *Annu Rev Ecol Syst* 35:347–374
- Eisenstein EM, Eisenstein D, Smith JC (2001) The evolutionary significance of habituation and sensitization across phylogeny: a behavioural homeostasis model. *Integr Phys Behav Sci* 36:251–265
- Eisner T (1981) Leaf folding in a sensitive plant: a defensive thorn-exposure mechanism. *Proc Natl Acad Sci USA* 78:402–404
- Esdin J, Pearce K, Glanzman DL (2010) Long-term habituation of the gill-withdrawal reflex in *Aplysia* requires gene transcription, calcineurin and L-type voltage-gated calcium channels. *Front Behav Neurosci* 4:181
- Fleurat-Lessard P, Bouche-Pillion S, Leloup C, Bonnemain J (1997) Distribution and activity of the plasma membrane H<sup>+</sup>-ATPase related to motor cell function in *Mimosa pudica* L. *Plant Physiol* 114:827–834
- Fromm J, Lautner S (2007) Electrical signals and their physiological significance in plants. *Plant Cell Environ* 30:249–257
- Gális I, Gaquerel E, Pandey SP, Baldwin IT (2009) Molecular mechanisms underlying plant memory in JA-mediated defence responses. *Plant Cell Environ* 32:617–627
- Giles AC, Rankin CH (2009) Behavioral and genetic characterization of habituation using *Caenorhabditis elegans*. *Neurobiol Learn Mem* 92:139–146
- Ginsburg S, Jablonka E (2009) Epigenetic learning in non-neural organisms. *J Biosci* 33:633–646
- Glanzman DL (2009) Habituation in *Aplysia*: the Cheshire cat of neuro-biology. *Neurobiol Learn Mem* 92:147–154
- Goodrich J, Tweedie S (2002) Remembrance of things past: chromatin remodeling in plant development. *Annu Rev Cell Dev Biol* 18:707–746
- Grissom N, Bhatnagar S (2009) Habituation to repeated stress: get used to it. *Neurobiol Learn Mem* 92:215–224
- Halling BD, Aracena-Parks P, Hamilton SL (2005) Regulation of voltage-gated Ca<sup>2+</sup> channels by calmodulin. *Sci STKE* 315:15. doi:10.1126/stke.3152005re15
- Han S-K, Wagner D (2013) Role of chromatin in water stress responses in plants. *J Exp Bot*. doi:10.1093/jxb/ert403
- Hemmi JM, Merkle T (2009) High stimulus specificity characterizes anti-predator habituation under natural conditions. *Proc R Soc B* 276:4381–4388
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. *Calif Agric Exp Stat Circ* 347:1–32
- Hoddinott J (1997) Rates of translocation and photosynthesis in *Mimosa pudica* L. *New Phytol* 79:269–272
- Inoue J (2008) A simple Hopfield-like cellular network model of plant intelligence. *Prog Brain Res* 168:169–174
- Jensen EL, Dill LM, Cahill JF Jr (2011) Applying behavioral-ecological theory to plant defense: light-dependent movement in *Mimosa pudica* suggests a trade-off between predation risk and energetic reward. *Am Nat* 177:377–381
- Karban R (2008) Plant behaviour and communication. *Ecol Lett* 11:727–739
- Karban R, Niiho C (1995) Induced resistance and susceptibility to herbivory: plant memory and altered plant development. *Ecology* 76:1220–1225
- Kawecki TJ (2010) Evolutionary ecology of learning: insights from fruit flies. *Popul Ecol* 52:15–25
- Kenzer AL, Ghezzi PM, Fuller T (2013) Stimulus specificity and dishabituation of operant responding in humans. *J Exp Anal Behav* 100:61–78
- Kim MC, Chung WS, Yun D-J, Cho MJ (2009) Calcium and calmodulin-mediated regulation of gene expression in plants. *Mol Plant* 2:13–21
- Kinoshita T, Jacobsen SE (2012) Opening the door to epigenetics in PCP. *Plant Cell Physiol* 53:763–765
- Krasne FB, Teshiba TM (1995) Habituation of an invertebrate escape reflex due to modulation by higher centers rather than local events. *Proc Natl Acad Sci USA* 92:3362–3366

- Ledón-Rettig CC, Richards CL, Martin LB (2013) Epigenetics for behavioral ecologists. *Behav Ecol* 24:311–324
- Lima SL (1998) Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Adv Study Behav* 227:215–290
- Limback-Stokin K, Korzus E, Nagaoka-Yasuda R, Mayford M (2004) Nuclear calcium/calmodulin regulates memory consolidation. *J Neurosci* 24:10858–10867
- Molinier J, Ries G, Zipfel C, Hohn B (2006) Transgeneration memory of stress in plants. *Nature* 422:1046–1049
- Moran N (2007) Osmoregulation of leaf motor cells. *FEBS Lett* 581:2337–2347
- Okano H, Hirano T, Balaban E (2000) Learning and memory. *Proc Natl Acad Sci USA* 97:12403–12404
- Pecinka A, Mittelsten Scheid O (2012) Stress-induced chromatin changes: a critical view on their heritability. *Plant Cell Physiol* 53:801–808
- Perisse E, Raymond-Delpech V, Neant I, Matsumoto Y, Leclerc C, Moreau M, Sandoz JC (2009) Early calcium increase triggers the formation of olfactory long-term memory in honeybees. *BMC Biol* 7:30. doi:10.1186/1741-7007-7-30
- Petrinovich L, Widaman KF (1984) An evaluation of statistical strategies to analyse repeated-measures data. In: Peeke HVS, Petrinovich L (eds) *Habituation, sensitization, and behaviour*. Academic Press, New York, pp 155–201
- Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, Coppola G, Geyer MA, Glanzman DL, Marsland S, et al. (2009) Habituation revisited: an updated and revised description of the behavioural characteristics of habituation. *Neurobiol Learn Mem* 92:135–138
- Reyes JC, Hennig L, Grisse W (2002) Chromatin-remodeling and memory factors. New regulators of plant development. *Plant Physiol* 130:1090–1101
- Roshchina VV (2001) *Neurotransmitters in plant life*. Science Publishers, Enfield
- Ruuhola T, Salminen JP, Haviola S, Yang S, Rantala MJ (2007) Immunological memory of mountain birches: effects of phenolics on performance of the autumnal moth depend on herbivory history of trees. *J Chem Ecol* 33:1160–1176
- Shepherd VA (2012) At the root of plant neurobiology. In: Volkov AG (ed) *Plant electrophysiology*. Springer, Berlin, pp 3–43
- Sung S, Amasino MR (2004) Vernalisation and epigenetics: how plants remember winter. *Curr Opin Plant Biol* 7:4–10
- Sztarker J, Tomsic D (2011) Brain modularity in arthropods: individual neurons that support “what” but not “where” memories. *J Neurosci* 31:8175–8180
- Thellier M, Lüttge U (2013) Plant memory: a tentative model. *Plant Biol* 15:1–12
- Thellier M, Desbiez MO, Champagnat P, Kergosien Y (1982) Do memory processes occur also in plants? *Physiol Plant* 56:281–284
- Thellier M, Le Sceller L, Norris V, Verdus MC, Ripoll C (2000) Long-distance transport, storage and recall of morphogenetic information in plants: the existence of a primitive plant “memory”. *C R Acad Sci III* 323:81–91
- Thompson RF (2009) Habituation: a history. *Neurobiol Learn Mem* 92:127–134
- Thorpe WH (1963) *Learning and instinct in animals*. Methuen, London
- Tomsic D, de Astrada MB, Sztarker J, Maldonado H (2009) Behavioral and neuronal attributes of short- and long-term habituation in the crab *Chasmagnathus*. *Neurobiol Learn Mem* 92:176–182
- Trewavas T (2003) Aspects of plant intelligence. *Ann Bot* 92:1–20
- Tseng AS, Levin M (2013) Cracking the bioelectric code: probing endogenous ionic controls of pattern formation. *Commun Integr Biol* 6:e22595
- Turner CH, Robling AG, Duncan RL, Burr DB (2002) Do bone cells behave like a neuronal network? *Calcif Tissue Int* 70:435–442
- Uehlein N, Kaldenhoff R (2008) Aquaporins and plant leaf movements. *Ann Bot* 101:1–4
- Verhoeven KJF, Jansen JJ, van Dijk PJ, Biere A (2010) Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol* 185:1108–1118
- Volkov AG, Carrell H, Adesina T, Markin VS, Jovanov E (2008) Plant electrical memory. *Plant Signal Behav* 3:490–492
- Wiel DE, Weeks JC (1996) Habituation and dishabituation of the proleg withdrawal reflex in larvae of the sphinx hawk, *Manduca sexta*. *Behav Neurosci* 110:1133–1147
- Yaish MW, Colasanti J, Rothstein SJ (2011) The role of epigenetic processes in controlling flowering time in plants exposed to stress. *J Exp Bot* 62:3727–3735
- Yang T, Poovaiah BW (2003) Calcium/calmodulin-mediated signal network in plants. *Trends Plant Sci* 8:505–512
- Yellen G (1998) The moving parts of voltage-gated ion channels. *Q Rev Biophys* 31:239–295