RESEARCH ARTICLE

Habituation in *Aedes aegypti* mosquito larvae is context specific

Ana Laura Pietrantuono[1], Maria B. Aguirre[2], Octavio A. Bruzzone*,[1] and Fernando J. Guerrieri*,[3],‡

ABSTRACT

Mosquito larvae live in water and perform a stereotyped escape response when a moving object projects its shadow on the water surface, indicating potential risk of predation. Repeated presentations of the shadow induce a decrease in the response as a result of habituation, a form of non-associative learning defined as the progressive and reversible decrease in response to a specific reiterative innocuous stimulus. Nevertheless, habituation can be context specific, which indicates an association between the context and the stimulus. The aim of this work was to study context specificity in habituation in mosquito larvae *Aedes aegypti*. Larvae were individually placed in Petri dishes positioned over black, white or black–white striped cardboard as background (visual context). Larvae were presented with a shadow produced by a cardboard square (training) over the course of 15 trials. After the 15th trial, the background was changed and the stimulus was presented once again (test). To analyse habituation in different contexts, we developed a series of learning curve models. We performed a Bayesian model selection procedure using those models and the data from the experiments to find which model best described the results. The selected model was a power law learning curve with six parameters (habituation rate; context-specific asymptotic habituation response, with one parameter per context, i.e. 3 parameters in total; response increase; and autocorrelation) describing the whole experimental setup with a generalised r^2 of 0.96. According to the model, a single habituation rate would indicate that habituation was independent of the context, whilst asymptotic habituation would be context specific. If the background was changed after training, there was an increase in response in the test, evincing context specificity in habituation.

KEY WORDS: Escape response, Insects, Non-associative learning, Vision

INTRODUCTION

Learning is crucial for any animal, as it allows adaptation of the individual's behaviour to changing environments (Giles and Rankin, 2009). Furthermore, being able to recognise and to differentiate among different kinds of stimuli reduces energy costs and time consumption (Scott, 2005). Habituation is a non-associative type of learning that allows filtering out of irrelevant stimuli. When an individual faces a repetitive stimulus without any

consequence, that stimulus becomes irrelevant. Consequently, a gradual decrease in its behavioural response occurs (Thompson and Spencer, 1966; Groves and Thompson, 1970; Giles and Rankin, 2009; Rankin et al., 2009; Rankin, 2009; Klein, 2012).

Habituation has often been considered among the simplest and most elementary forms of learning. It has been documented in a wide diversity of animals, both vertebrates and invertebrates. Among vertebrates, habituation has been studied in horses (e.g. Christensen et al., 2010), rats (e.g. Askew, 1970), birds (e.g. Dong and Clayton, 2009), fishes (e.g. Best et al., 2008), rabbits (e.g. Whitlow, 1975), cats (e.g. Groves and Thompson, 1970), dogs (e.g. Pullen et al., 2012) and humans (e.g. Dycus and Powers, 1997). Among invertebrates, habituation has been studied in the nematode *Caenorhabditis elegans* (e.g. Rankin, 2000), molluscs (e.g. Pinsker et al., 1970; Carew and Kandel, 1973; Bailey and Chen, 1983; Fischer et al., 2011), crabs (e.g. Brunner and Maldonado, 1988; Lozada et al., 1990; Tomsic et al., 1998, 2009; Hemmi and Tomsic, 2012; Raderschall et al., 2011) and insects (Tachind fly *Drino bohemica*: Monteith, 1963; honey bee *Apis mellifera*: Braun and Bicker, 1992; Gerber and Menzel, 2000; fruit fly *Drosophila melanogaster*: Glanzman, 2011; Soibam et al., 2013; Twick et al., 2014). More recently, habituation-like behaviour has been reported in the myxomycete slime mould *Physarum polycephalum* (Boisseau et al., 2016).

The adult mosquito *Aedes aegypti* is the vector of several human diseases such as dengue, yellow fever, Chikungunya, Zika and Mayaro viruses (Marklewitz and Junglen, 2019). Pre-imaginal stages of this holometabolous insect, i.e. from egg to pupa, develop in clean and calm bodies of water. When confronted with a moving visual stimulus above the water surface (e.g. a shadow), mosquito larvae and pupae perform a stereotyped escape response (fast movements with the abdomen allowing the animal to dive; Holmes, 1911; Thomas, 1949; Leftwich, 1954; Mellamby, 1958). The escape response causes significant energy expenditure, affecting survival and fertility (Timmermann and Briegei, 1993; Lucas and Romoser, 2001). Cognitive abilities of mosquitoes in their pre-imaginal stages have been barely studied. Recently, Baglan et al. (2017) reported habituation leaving a mnesic trace lasting for 1 h in mosquito larvae of *A. aegypti*. Analogous to the work performed by Brunner and Maldonado (1988) in the crab *Neohelice granulata* (*Chasmagnathus granulatus*), Baglan et al. (2017) quantified the reduction of the escape response to the shadow projected by an object moving over the surface of the water (named by the authors as a 'visual danger stimulus') subsequent to repeated presentations.

According to Chilaka et al. (2012), it is particularly important to study learning and memory in mosquitoes and other insects that transmit human diseases because their behaviour determines their success as disease vectors (McCall and Kelly, 2002; Alonso and Schuck-Paim, 2006; Bouyer et al., 2007; Vinauger et al., 2014, 2016). Therefore, behavioural studies in mosquitoes must take into account all the developmental stages, as larval performance has an impact on their adult success (McCall and Eaton, 2001; Kaur et al., 2003). So far, studies on cognitive ability in pre-imaginal stages remain scarce, as mentioned above.

[1]IFAB-CONICET-INTA EEA Bariloche, Modesta Victoria 4450. San Carlos de Bariloche (8400), Río Negro, Argentina. [2]CONICET-FuEDEI Simón Bolívar 1559, Hurlingham (T4001MVB), Buenos Aires, Argentina. [3]IRBI- Institut de Recherche sur la Biologie de l'Insecte UMR 7261, CNRS – Université de Tours, Av Monge, Parc de Grandmont, 372000 Tours, France.
*These authors contributed equally to this work

‡Author for correspondence (fernando.guerrieri@univ-tours.fr)

A.L.P., 0000-0002-0073-4026; M.B.A., 0000-0002-3773-8805; O.A.B., 0000-0003-2358-1963; F.J.G., 0000-0001-9872-9067

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Habituation is traditionally understood as a non-associative form of learning. However, during habituation, animals can integrate other stimuli constantly present in their environment. Those stimuli are together known as the context, i.e. the surrounding conditions where learning takes place (Myers and Gluck, 1994). Contextual stimuli can be associated with habituation stimuli. When such an association exists, it determines the contextual specificity of habituation. Context dependency in habituation was demonstrated in the nematode *C. elegans* (Rankin et al., 1990; Bernhard and van der Kooy, 2000; Rankin, 2000) and in the sea-hare *Aplysia californica* (Pinsker et al., 1970; Carew and Kandel, 1973). In the crab *N. granulata*, it was shown that when the context was changed, habituation to a shadow passing over the crab was reversed (Hermitte et al., 1999).

The fact that context dependency of habituation has been observed in diverse organisms led us to hypothesise that this is a generalised phenomenon. Consequently, we asked whether habituation in the larvae of *A. aegypti* was context-dependent as well.

In the present work, we studied whether mosquito larvae were able to associate visual contextual stimuli with the stimulus inducing habituation. To that end, mosquito larvae were individually trained in a Petri dish positioned over a set visual pattern (the context), with a shadow stimulus presented above. Once habituation was induced, the context was changed and the shadow stimulus was presented again. Context dependency of habituation would be evinced if the larvae performed the escape response.

MATERIALS AND METHODS
Experimental subjects
Fourth-instar larvae of *Aedes aegypti* Bora strain were used in this work. Mosquitoes were reared in a climate-controlled room at 25°C with 12 h:12 h light:dark cycle. Larvae were obtained from eggs provided by the Laboratoire d'Insectes Nuisibles (MIVEGEC-IRD, Montpellier, France). Eggs were put in small plastic containers (12×6×6 cm l×w×h) with dechlorinated tap water. Larvae were fed with shrimp food (JBL Novo Prawn, Neuhofen, Germany; www.jbl.de). Under these conditions, larvae reached their fourth instar after 5 days and moulted into pupae 5 days later, i.e. it took around 10 days from hatching to pupal moult. In order to avoid using pharate pupae, we did not use larvae older than 8 days. All the animals were reared and treated according to the rules and regulations on ethics applied in the European Union.

Apparatus
The protocol used in the experiments was adapted from Baglan et al. (2017). The experiments were performed in a different room at 24±1° C. Each larva was individually placed into a Petri dish (3 cm diameter and 1.2 cm height) filled with dechlorinated tap water, and the Petri dish was placed on a table directly under a spot light. A square of cardboard was positioned under each Petri dish to provide the visual context. The cardboard was painted with one of the following three patterns: 100% white, 100% black or striped black and white (each stripe was 1 cm wide; black and white each covered 50% of the surface). The Petri dishes were visually isolated from each other by opaque walls (30 cm in height and width). To avoid any disruption caused by vibrations, each Petri dish was placed on a foam block.

The visual stimulus was presented by a mechanical arm; namely, a wooden stick, 18 cm long, with a 3×3 cm square of cardboard at the end. The cardboard square projected a shadow stimulus to be perceived by the naive larva; this signal might be similar to the visual stimulus provided by an aerial predator in nature. Consequently, the naive larva performed the escape response. We used a servomotor associated with a control board (Arduino Uno; http://www.arduino.cc)

to automatically deliver the habituation stimulus. The angular speed of the stimulus was 315.8 deg s^{-1} at 4.8 V.

Habituation protocol
The protocol of habituation included three phases: acclimatation, training and test. During acclimatation, each larva was deposited in a Petri dish under the spot light and rested for 30 min. Over the course of the training phase, the mechanical arm projected its shadow on the Petri dish for 1.5 s, with an inter-trial interval of 5 min. Training was carried out for a total duration of 1 h and 15 min. The visual stimulus was displayed 15 times, i.e. 15 trials. In every trial, the response of the larva was recorded, i.e. escape response=1, no response=0. The escape response was evinced by fast movements of the abdomen, allowing the animal to dive (see the video in Baglan et al., 2017). A decrease in the escape response over the course of the training phase would indicate habituation (see Baglan et al., 2017). Once the training phase was completed, the Petri dish was very gently raised to avoid any disturbance that could provoke dishabituation (Baglan et al., 2017). The piece of cardboard beneath it (the context) was removed and either replaced by another one or put back, in experimental or control groups, respectively; larvae were then left to rest for 10 min. The test phase then occurred, consisting of one presentation of the visual stimulus.

Experimental design
Three different types of background (white, black or striped) were used as context (i.e. the stimulus that was present during the whole training phase; Nadel and Willner, 1980), for both training and test. All possible combinations represented nine independent experimental groups; each group included 30 replicates. In total, 270 individuals were included (Fig. 1).

Data analyses
The data analyses were performed using a Bayesian model selection procedure on a series of mathematical models describing the habituation process, starting with the simplest model and increasingly adding complexity until the information criterion used worsened or the full model with all the parameters was reached.

Learning model
A series of Hidden Markovian models (Eddy, 2004), based on the power law of practice (Snoddy, 1926), was used to explain the dynamics of learning. Our explanations were based on the experimental data. According to this approach, learning does not occur at a constant speed but is slowed down over the course of the habituation trials. In the beginning, learning occurs faster; however, further improvements are more difficult. Power law models propose that learning decreases linearly with the logarithm of the number of practice trials taken, thus producing an exponential decay, in which the response tends asymptotically towards a minimum value (generally zero). In this case, the escape response is expected to decrease as the larva is subjected to successive tests until it approaches a minimum baseline value (see Results for details).

The model we applied is well known and it has been widely tested in human learning (see Fitts and Posner, 1967; Anderson, 1982; Card et al., 2017). More recently, Evans et al. (2018) developed Bayesian versions of this model.

The basic structure of the model is:

$$R_t = (1 - h)R_{(t-1)}, \tag{1}$$

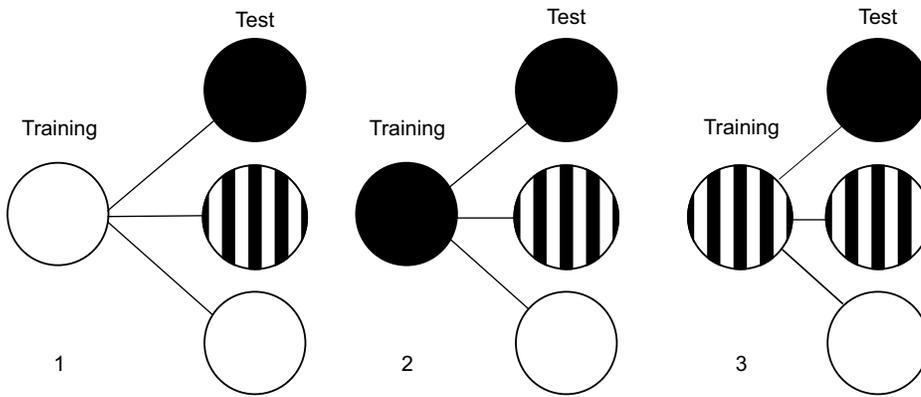$$R_e = (1 - R_a)R_t + R_a, \tag{2}$$

Fig. 1. Experimental design. Three backgrounds were used as context in the training and test phases: (1) white, (2) black and (3) striped. Each visual background was replaced between the end of the training phase and the test phase. The visual background in the test was changed in the experimental groups, i.e. nine treatments were performed (*n*=30 in each group). For the control, the background was replaced with the same background.

where R_t is the expected response at trial t, and habituation is expressed as a response that decreases exponentially at a rate h towards zero. As the final response can be greater than zero, the response R_t is rescaled so that instead of tending asymptotically to zero, it tends towards a value called R_a, which would be the average response of the experimental subjects after an infinite number of trials; the rescaled value is called R_e.

Once the tests were finished, the habituation could decrease or not, according to the response d during the test. The full development of the model is shown in the Appendix (see Table A1).

Statistical methods

The main objective of the analyses was to define whether or not the model parameters differed among treatments (test settings, and their combinations), and to what degree. Once we defined which parameters were specific to the treatments and which parameters were general to the learning model, the values of the parameters were calculated.

The model describing learning is non-linear. Hence, the parameters could interact with each other in many possible ways. As a consequence, we used a process of selection of models through information indices to obtain an optimal model instead of simple statistical tests of the null hypothesis. These parameters would be treatment specific only in the event that a given parameter varied between treatments, and would be common to all in cases where the parameter did not vary between treatments.

Consequently, the data were analysed using a stepwise model selection procedure under a full Bayesian approach. We proposed a series of explanatory models that were generated from the null model, becoming more complex by adding parameters to each step, thus increasing complexity. The development of these models continued until the model complexity exceeded the increase in complexity caused in the model by adding a new parameter. The deviance information criterion (DIC; Gelman et al., 2004) was used as a decision rule for accepting/rejecting the proposed models. When a newly more complex model was rejected, because the DIC increased, the procedure stopped, and we kept the last selected model as the explanatory model.

The distribution of parameters of the models was calculated using the Markov Chain Monte Carlo (MCMC) algorithm with the pymc module for stochastic modelling in Python version 2.3.8 (Patil et al., 2010).

Non-informative uniform *a priori* distributions between 0 and 1 were used for all the parameters, except the autocorrelation coefficient, which was between −1 and 1. A total of 200,000 iterations of the MCMC were used, of which the first 100,000 were

discarded as a burn-in, and the remaining were used to estimate the *a posteriori* distributions.

Using a Bayesian approach provides a more straightforward way to fit and select among a great number of models, unlike frequentist statistical methods, and the same approach has already been used by ecologists in animal behaviour studies (Ellison, 2004; McNamara et al., 2006; Pietrantuono et al., 2015, 2017).

Additionally, the generalised coefficient of determination (GCD) for binary data, according to Cox and Snell (1989) and Magee (1990), was calculated as a measure of the fitness of each of the proposed models to the data of the experiments.

RESULTS

Model of *A. aegypti* habituation

A total of 31 models (plus null model) were developed. Through the model selection process, we obtained a model that, according to the information criteria used (minimum DIC), was optimal in terms of explanatory power and complexity (model no. 18, Table A1). The resulting model contained six parameters (h, d_c, R_{aw}, R_{ab}, R_{as} and ϕ), detailed in Table A1, and its explanatory power in terms of GCD was 0.9611. This means that this model is capable of explaining 96% of the variance of the study data. As explained above, all parameters that differed among test contexts, testing or their interactions, were included in the model as separate parameters (where different contexts are indicated by subscript j), while the parameters that did not differ were replaced by a single common parameter for all treatments.

The model itself is described in Eqn A9 of the Appendix, with one parameter R_a (asymptotic response) depending on the context [one parameter per context, w (white), b (black), s (striped), resulting in three asymptotic responses: R_{aw}, R_{ab}, R_{as}]; the increase in response occurred only in the case of a change of the context, but not for the context itself, so it is called d_c (here, the subscript c is used to denote the change of context) and the parameters h (the habituation rate) and ϕ (the autoregressive coefficient) are independent of any context. In this way, the final model of learning by habituation for *A. aegypti* was:

$$R_{tj} = \delta_j(1-h)^t + \varphi(O_{i_{(t-1)}} - R_{O_{i(t-1)}}) + (1-\delta_j)(d_c(j \neq k)), \tag{3}$$

where in Eqn A2 of the Appendix, the response decays exponentially to R_a instead of zero as the conditioning trials are performed, so the final observed response is R_{etj}:

$$R_{etj} = (1-R_{aj})R_{tj} + R_{aj}, \tag{4}$$

Table 1. Estimated parameters of the selected model

Parameter	Symbol	Context	Mean estimation and 95% CI
Habituation rate	h	All	0.306 [0.266–0.351]
Response increase	d_c	Different	0.149 [0.098–0.200]
Asymptotic response	R_{aw}	White	0.270 [0.240–0.296]
	R_{ab}	Black	0.324 [0.293–0.358]
	R_{as}	Striped	0.278 [0.248–0.305]
Autoregressive coefficient	ϕ	All	0.311 [0.283–0.338]

Mean estimated values of the response in the training phase are shown for each parameter from the selected model, with the 95% credibility interval (CI) in brackets. Subscripts w, b and s represent the white, black and striped backgrounds, respectively.

where j and k indicate the trial and test context, respectively; both can take the w, b or s values, δ is a Kronecker delta which indicates that the insect is in trial (δ=1) or test (δ=0) context, and O_i is the observed response for the individual i at trial t.

The parameters calculated for this model (see Table 1) show that, on the one hand, the habituation rate (h) was the same for all contexts, whilst, on the other hand, the asymptotic response (R_a) depended on the context, being similar between the white and striped background, but higher with the black background. As explained in Materials and Methods, the asymptotic response had an inverse relationship with habituation, so the white and striped backgrounds favoured habituation in the long term. As can be seen in Fig. 2, the final responses with the white and striped backgrounds were similar, and the response was greatest with the black background, with similarly sloped curves in all cases.

Finally, autocorrelation was strong, as the autoregressive coefficient (ϕ) was estimated to be 0.3 (see Table 1) and could alter the results in unpredictable ways if not taken into account.

Response levels in training and test phases

The results of our experiments evinced that the insects included in this study had a power law-type learning curve, in which the response decreased to a level that was higher than zero but lower than the initial response level. Over the course of the 15 training trials, the response to the visual stimulus decreased in all cases (Fig. 2). Therefore, habituation was observed independently of the visual background (context).

Some parameters of the learning curves were shared across all the contexts, while others were context specific (Table 1). All the backgrounds shared the same habituation rate (h), but differed in their asymptotic responses (R_a); as a consequence, the speed of habituation was similar regardless of the background (visual context).

The parameter d (response increase), which was only present (i.e. different from zero) in the case of a change of background, indicates that in the test phase, the insects retained the same R_a of escape/
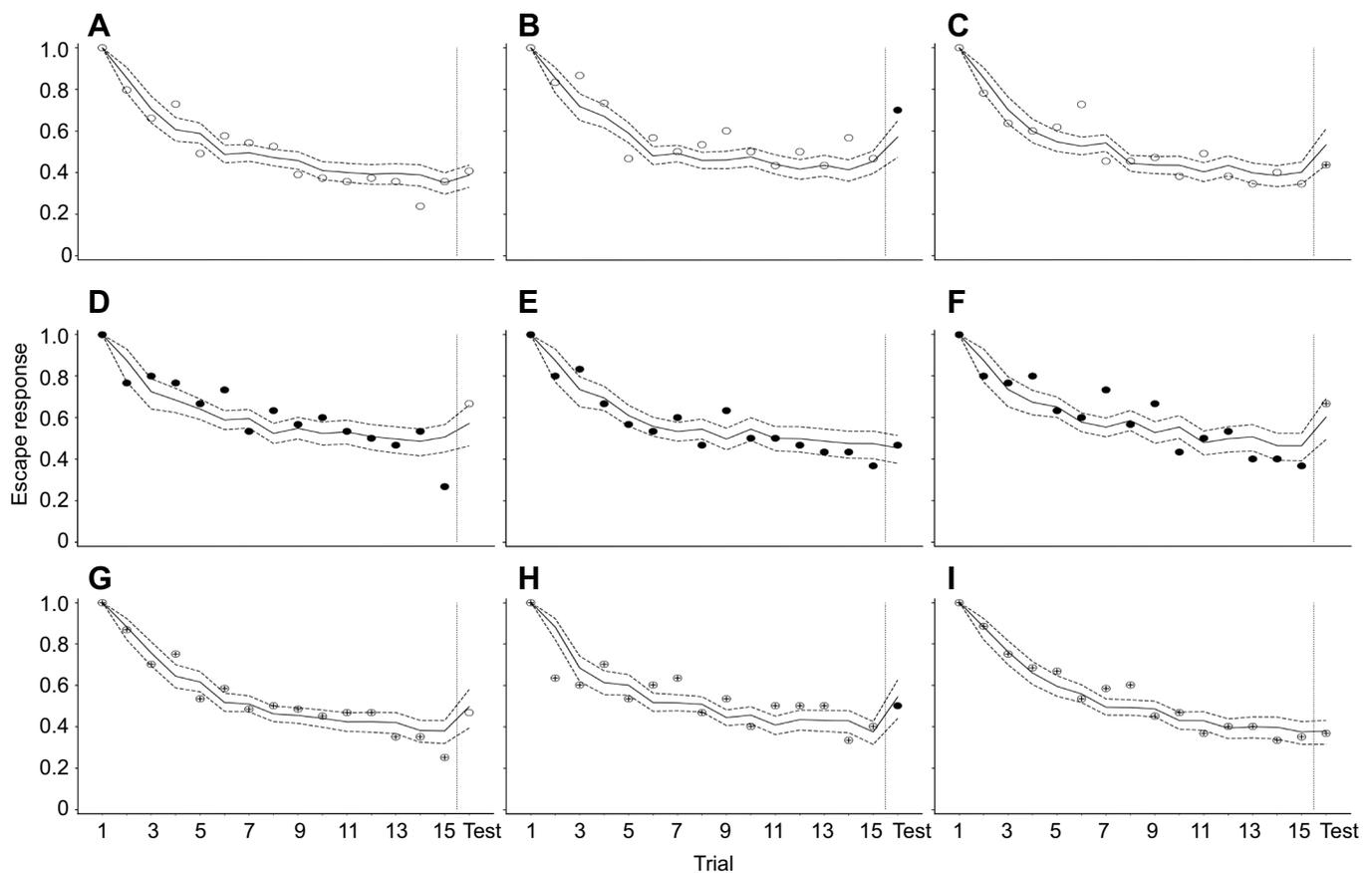

Fig. 2. Response levels in training and test phases. The escape response is expressed as the number of larvae performing escape responses on presentation of the visual stimulus out of the total number of assayed individuals (*n*=30) for each of the nine experimental groups (A,E,I: using the same background; B–D,F–H, with a change in background). The *x*-axis indicates the 15 training trials plus the test. White, black and stripped backgrounds are indicated by white, black and crossed circles, respectively. Solid lines indicate the *a posteriori* estimated mean responses and dashed lines indicate the 95% credibility interval. The vertical line separates training from test values.

response level as at the end of the training phase when using the same background, as can be seen in Fig. 2A,E,I. In the case of a change in the background between training and test, the response increased. This increase in response may be attributable to the change in background (Fig. 2B–D,F–H).

DISCUSSION

n all the visual backgrounds applied in the present work, *A. aegypti* larvae could be habituated to a visual stimulus. In all the cases, the decrease in response due to habituation did not depend on the visual background (context), which was evinced by the fact that the slopes among all the learning curves did not differ. However, the habituation level was lowest when larvae were trained with the black visual context. In all cases, changing the visual context between the training and the test phases induced an increase in the response in the test, thus proving that habituation was context dependent.

As mentioned above, larvae could be habituated in all contexts. At the same time, we observed that, over the course of the training phase, a decrease in escape response occurred at the same rate in the first five trials, i.e. the slopes of the acquisition curves did not differ. In subsequent trials, behaviour differed according to the visual context. When larvae were trained with the black context, the slope decreased from the 6th trial onwards, and the response reached its asymptote at a 10% greater level than in the cases in which larvae were trained with either a striped or a white context. Therefore, habituation performance depended on the visual context. In particular, the larvae exhibited their best learning performance when trained with lighter visual contexts. In those cases, the contrast between the shadow of the moving cardboard square was greater when projected on the white than on the black surface. The lower habituation level observed with the black context could be due to the contrast between the shadow and the background being lower than in the other two contexts. Quantification of the effects of contrast on habituation level should be carried out in future experiments. For example, larvae could be trained on different solid greyscale or striped backgrounds varying the width of the stripes or the white/black area ratio.

In our study, mosquito larvae that had habituated to the visual stimulus on a white background no longer responded to the visual stimulus when tested on a white background, whilst they responded to the same stimulus when tested on a black background. Conversely, the same stimulus presented on a different visual background, i.e. a different context, was perceived as a novel stimulus inducing larvae to escape. Context specificity led us recognise that there must have been some kind of associative component of habituation between the context and the visual stimulus, as has been observed in previous work in crabs (Tomsic et al., 1998; Hermitte et al., 1999; Pereyra et al., 2000).

Thanks to the use of Markovian models combined with Bayesian statistics, it was possible to make an accurate description of the mosquito learning curve. Likewise, it was possible to formulate parameters with great biological significance, improving what has been achieved so far using generic statistical models such as those that are most commonly used to analyse habituation experiments. In this work, we calculated the rate, and the final habituation value, the increase in response produced at the end of the training procedure, and its dependency on the context, all corrected by autocorrelation, using an autoregressive (AR) term. Therefore, we suggest the use of this methodological approach to analyse this type of experiment in the future, which significantly increases the amount of information in a study, while maintaining the same design and experimental effort, using the same laboratory equipment.

Our results contribute to the so far scarcely explored learning ability of the mosquito larvae of *A. aegypti*, in particular, the ability to associate a constantly present visual stimulus (the context) with a moving object. Are mosquito larvae capable of associative learning? The question remains open.

Comprehensive understanding of the pre-imaginal behaviour of mosquitoes – in particular, the behavioural plasticity – will certainly allow us to better understand their adaptability to different environments. This is an important piece of the jigsaw puzzle of integrative pest control and environmental conservation.

APPENDIX

Model description and selection

A series of hidden Markovian models (Eddy, 2004) is proposed to explain habituation. The hidden Markovian model assumes that habituation is a stochastic process in which, as in any Markovian process, future states depend only on the current state. Here, the state variable R (the expected response, i.e. the proportion of times that a given animal will respond to the presented stimulus) cannot be observed directly at an individual level (so the model is hidden), but can be inferred after observing the response of the experimental animal after each test. If R is a proportion, it is restricted to values between 0 and 1; therefore, R as a function of time was modelled as a power law learning curve as follows:

$$R_t = (1 - h)R_t - 1, \qquad (A1)$$

where R_t is the expected response at trial t and h is the habituation rate, with h being $0 \leq h \leq 1$; so, if h is equal to 0, there is no habituation ($R_t = R_{t-1}$), and if h is 1, the maximum habituation is reached after only one trial ($R_t = 0$), independently of the value of R_{t-1}. In this model, R decays exponentially to 0 after an infinite number of trials. However, it is possible that the asymptotic response will be different from 0: after a large number of trials, some animals will still be responding positively to the stimulus. We therefore use R_a for that asymptotic response (a lower asymptotic response means higher habituation). Now, Eqn A1 is modified as follows:

$$R_e = (1 - R_a)R_t + R_a, \qquad (A2)$$

where R_e is the expected response, which is R_t multiplied by $1 - R_a$ plus R_a, so the response decays exponentially to R_a instead of 0 as the conditioning trials are performed.

The observed increase in response after the training phase finished is also assumed to be a function with exponential decay, but now in the reverse direction, so R_t increases asymptotically to 1. Therefore, the model is modified as:

$$R_t = \delta(1 - h)R_{t-1} + (1 - \delta)(1 - (1 - d)R_{t-1})$$
$$+ \varphi(O_{i(t-1)} - R_{e(t-1)}), \qquad (A3)$$

where δ is a Kronecker delta, with a value of 1 if the animal is in a conditioning trial and 0 otherwise, and d is the response in the test. So, the first term (exponential decay in response) is active during the conditioning trials, and the other is 0, whereas during the test phase the first term is 0, and the second is active, giving an exponential increase. Finally, to compensate for autocorrelation in the response of the same individual used in the trials, an autoregressive term (AR) is added. This term attempts to correct for the increase or decrease in the expected response, when the experimental animal gives a positive or

Table A1. Models proposed to explain the behaviour of the assayed larvae

Model no.	Model	Variables	Variables dependent on context	No. of parameters	DIC values	GCD
0	Null model (*Hr*=0)	R_a	–	1	7967.14	–
1	*Hr* (*Ra*=0)	*h*	–	1	7786.11	0.4885
2	*Hr*+*Ra*	*h*, R_a	–	2	7607.97	0.7375
3	*Hr* depending on context (*Ra*=0)	*h*	*h*	3	7767.95	0.5288
4	*Hr* depending on context+*Ra*	*h*, R_a	*h*	4	7594.21	0.7543
5	Model 3+*Ra* depending on context	*h*, R_a	R_a	4	7590.99	0.7572
6	Model 4+*Ra* depending on context	*h*, R_a	*h*, R_a	6	7593.31	0.7587
7	Pure *Ra* model (*h*=0)	R_a, φ	–	2	7387.26	0.8841
8	*Hr*+*Ra* (*Ra*=0)	*h*, φ	–	2	7244.82	0.9316
9	Model 3+AR	*h*, R_a, φ	–	3	7128.27	0.9559
10	Model 4+AR	*h*, R_a, φ	*h*	5	7127.37	0.9567
11	Model 5+AR	*h*, R_a, φ	R_a	5	7121.10	0.9577
12	Model 6+AR	*h*, R_a, φ	*h*, R_a	7	7122.59	0.9581
13	Model 9+response-increase	*h*, R_a, φ, *d*	–	4	7116.83	0.9581
14	Model 10+response-increase	*h*, R_a, φ, *d*	*h*	6	7114.82	0.9590
15	Model 11+response-increase	*h*, R_a, φ, *d*	R_a	6	7109.93	0.9597
16	Model 12+response-increase	*h*, R_a, φ, *d*	*h*, R_a	8	7111.68	0.9601
17	Model 14+response-increase only when changing context $j \neq k$	*h*, R_a, φ, *d*	*H*, $d(j \neq k)$	6	7105.73	0.9603
18	**Model 15+response-increase only when changing context $j \neq k$**	***h*, R_a, φ, *d***	**R_a, $d(j \neq k)$**	**6**	**7100.52***	**0.9611**
19	Model 16+response-increase only when changing context $j \neq k$	*h*, R_a, φ, *d*	*h*, R_a, $d(j \neq k)$	8	7103.38	0.9613
20	Model 14+response-increase depending on same context ($j=k$) or change of context ($j \neq k$)	*h*, R_a, φ, *d*	*h*, $d(j \neq k)(j=k)$	7	7107.45	0.9604
21	Model 15+response-increase depending on same context ($j=k$) or change of context ($j \neq k$)	*h*, R_a, φ, *d*	R_a, $d(j \neq k)$ $(j=k)$	7	7102.39	0.9611
22	Model 16+response-increase depending on same context ($j=k$) or change of context ($j \neq k$)	*h*, R_a, φ, *d*	*h*, R_a, $d(j \neq k)(j=k)$	9	7105.18	0.9613
23	Model 14+response-increase depending on habituation context (j)	*h*, R_a, φ, *d*	*h*, $d(j)$	8	7111.62	0.9601
24	Model 15+response-increase depending on habituation context (j)	*h*, R_a, φ, *d*	R_a, $d(j)$	8	7109.29	0.9604
25	Model 16+response-increase depending on habituation context (j)	*h*, R_a, φ, *d*	*h*, R_a, $d(j)$	10	7112.76	0.9605
26	Model 14+response-increase depending on test context (j)	*h*, R_a, φ, *d*	*h*, $d(k)$	8	7116.30	0.9594
27	Model 15+response-increase depending on test context (j)	*h*, R_a, φ, *d*	R_a, $d(k)$	8	7111.59	0.9601
28	Model 16+response-increase depending on test context (k)	*h*, R_a, φ, *d*	*h*, R_a, $d(k)$	10	7114.02	0.9603
29	Model 14+response-increase the combination of both habituation and test context (j, k)	*h*, R_a, φ, *d*	*h*, $d(j,k)$	14	7110.51	0.9620
30	Model 15+response-increase the combination of both habituation and test context (j, k)	*h*, R_a, φ, *d*	R_a, $d(j,k)$	14	7107.77	09623
31	Model 16+response-increase the combination of both habituation and test context (j, k)	*h*, R_a, φ, *d*	*h*, R_a, $d(j,k)$	16	7110.34	0.9625

Models proposed in the Bayesian selection process, its parameters and values of the deviation information criterion (DIC), and generalised coefficient of determination for logistic distribution (GCD), according to Cox and Snell (1989) and Magee (1990). Bold indicates the selected model with the lowest DIC value. Asterisks indicate the selected model. The null model does not have a coefficient of determination, because it is used to calculate this coefficient for the other models. The model terms are abbreviated as follows: *Hr* (symbol *h*), habituation rate; *Ra* (symbol R_a), asymptotic response; AR, autoregressive term (symbol φ). For definitions of variables, see Appendix.

negative response to the stimulus:

$$R_t = \delta(1-h)R_{t-1} + (1-\delta)(1-(1-d)R_{t-1}) + \varphi(O_{i(t-1)} - R_{e(t-1)}), \quad (A4)$$

where φ is the autocorrelation coefficient, $O_{i(t-1)}$ is the observed response of the animal i in the previous trial, and $R_{e(t-1)}$ is the expected response in the previous trial.

Then, it is possible to simplify, given that all the animals have the same initial conditions at $t=0$, with $R_t=0=1$, and only one test trial is performed. The full model for any trial is set as follows:

$$R_t = \delta(1-h)^t + \varphi(O_{i(t-1)} - R_{O_{i(t-1)}}) + (1-\delta)d. \quad (A5)$$

If the response of the experimental animal is context dependent, Eqn A5 could be modified as follows:

$$R_{tj} = \delta(1-h_j)^t + \varphi(O_{i(t-1)} - R_{O_{i(t-1)}}) + (1-\delta_j)d, \quad (A6)$$

where as in Eqn A2, the response decays exponentially to R_a instead of 0 as the conditioning trials are performed, so the final observed response is R_{etj}:

$$R_{etj} = (1-R_{aj})R_{tj} + R_{aj}, \quad (A7)$$

where subscript j corresponds to the context in which the trial was performed. Intermediate models between Eqns A1 and A2 were possible, with some of the parameters R_a, h and d being context dependent. Furthermore, under this experimental design, the test phase could be performed in a different context from the training phase, so Eqn A2 could be modified to add another subscript called k, which is the context in which the trial stage was performed.

Then, the variable d becomes dependent in two different contexts j and k. If j and k represent the total number of contexts used in the training and test phases respectively, the d variable follows j parameters if it depends on habituation context, and k parameters if it only depends on test phase context, and $j \times k$ parameters if it depends on both contexts. Finally, there is the possibility that the response increase does not depend on the context itself, but on the
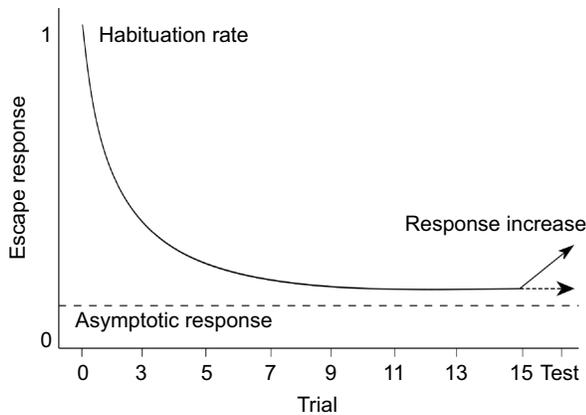
Fig. A1. Conceptual model of the habituation learning curve. Graph showing the habituation curve proposed in this study. Habituation is assumed to produce a decreasing mean response that decays exponentially at a given rate until the trials are completed. The initial response is 1, and it decays to an asymptotic value greater than 0, shown by the dashed line.

change of context:

$$R_{tj} = \delta(1 - hj)^t + \varphi(O_{i(t-1)} - R_{O_{i(t-1)}}) \\ + (1 - \delta_j)(d_{\mathrm{e}}(j = k) + d_{\mathrm{c}}(j \neq k)), \tag{A8}$$

where the asymptote is greater than 0, as in Eqn A2, so the final observed response is $R_{\mathrm{e}tj}$:

$$R_{\mathrm{e}tj} = (1 - R_{aj})R_{tj} + R_{aj}. \tag{A9}$$

Thus, there are two possible response increase parameters, one (d_{e}) that corresponds to the expected response increase, if the test context is the same as the conditioning context ($j=k$), and a second ($j \neq k$) that corresponds to the expected increase in response if the test context is different from the conditioning context (d_{c}) (see Fig. A1). For model selection procedure by means of the deviation information criterion (DIC), see Table A1.

References

Alonso, W. J. and Schuck-Paim, C. (2006). The 'ghosts' that pester studies on learning in mosquitoes: guidelines to chase them off. *Med. Vet. Entomol.* **20**, 157-165. doi:10.1111/j.1365-2915.2006.00623.x

Anderson, J. R. (1982). Acquisition of cognitive skill. *Psychol. Rev.* **89**, 369. doi:10.1037/0033-295X.89.4.369

Askew, H. R. (1970). Effects of stimulus intensity and intertrial interval on habituation of the head-shake response in the rat. *J. Comp. Physiol. Psychol.* **72**, 492-497. doi:10.1037/h0029745

Baglan, H., Lazzari, C. and Guerrieri, F. (2017). Learning in mosquito larvae (*Aedes aegypti*): habituation to a visual danger signal. *J. Ins. Physiol.* **98**, 160-166. doi:10.1016/j.jinsphys.2017.01.001

Bailey, C. H. and Chen, M. (1983). Morphological basis of long-term habituation and sensitization in *Aplysia*. *Science* **220**, 91-93. doi:10.1126/science.6828885

Bernhard, N. and van der Kooy, D. (2000). A behavioral and genetic dissection of two forms of olfactory plasticity in *Caenorhabditis elegans*: Adaptation and habituation. *Learn. Mem.* **7**, 199-212. doi:10.1101/lm.7.4.199

Best, J. D., Berghmans, S., Hunt, J. F. G., Clarke, S. C., Fleming, A., Goldsmith, P. and Roach, A. G. (2008). Non-associative learning in larval Zebrafish. *Neuropsychopharmacology* **33**, 1206-1215. doi:10.1038/sj.npp.1301489

Boisseau, R. P., Vogel, D. and Dussutour, A. (2016). Habituation in non-neural organisms: evidence from slime moulds. *Proc. R. Soc. B* **283**, 20160446. doi:10.1098/rspb.2016.0446

Bouyer, J., Pruvot, M., Bengaly, Z., Guerin, P. M. and Lancelot, R. (2007). Learning influences host choice in tsetse. *Biol. Lett.* **3**, 113-117. doi:10.1098/rsbl.2006.0578

Braun, G. and Bicker, G. (1992). Habituation of an appetitive reflex in the honeybee. *J. Neurophysiol.* **67**, 588-598. doi:10.1152/jn.1992.67.3.588

Brunner, D. and Maldonado, H. (1988). Habituation in the crab *Chasmagnathus granulatus*: effect of morphine and naloxone. *J. Comp. Physiol. A* **162**, 687-694. doi:10.1007/BF01342643

Card, S. K., Moran, T. P. and Newell, A. (2017). *The Psychology of Human-Computer Interaction*, 1st edn. Kindle edn. Boca Ratón, FL, USA: CRC Press.

Carew, T. J. and Kandel, E. R. (1973). Acquisition and retention of long-term habituation in *Aplysia*: correlation of behavioral and cellular processes. *Science* **182**, 1158-1160. doi:10.1126/science.182.4117.1158

Chilaka, N., Perkins, E. and Tripet, F. (2012). Visual and olfactory associative learning in the malaria vector *Anopheles gambiae* sensu stricto. *Malar. J.* **11**, 1-27. doi:10.1186/1475-2875-11-27

Christensen, J. W., Rundgren, M. and Olsson, K. (2010). Training methods for horses: habituation to a frightening stimulus. *Equine Vet. J.* **38**, 439-443. doi:10.2746/042516406778400574

Cox, D. R. and Snell, E. J. (1989). *Analysis of Binary Data*. Chapman & Hall, London.

Dong, S. and Clayton, D. F. (2009). Habituation in songbirds. *Neurobiol. Learn. Mem.* **92**, 183-188. doi:10.1016/j.nlm.2008.09.009

Dycus, W. A. and Powers, A. S. (1997). Eyeblink cross-habituation between tactile and acoustic system in humans. *Psychobiology* **28**, 507-514.

Eddy, S. R. (2004). What is a hidden Markov model?. *Nat. Biotechnol.* **22**, 1315-1316. doi:10.1038/nbt1004-1315

Ellison, A. M. (2004). Bayesian inference in ecology. *Ecol. Lett.* **7**, 509-520. doi:10.1111/j.1461-0248.2004.00603.x

Evans, N. J., Brown, S. D., Mewhort, D. J. K. and Heathcote, A. (2018). Refining the law of practice. *Psychol. Rev.* **125**, 592. doi:10.1037/rev0000105

Fischer, T. M., Jacobson, D. A., Counsell, A. N., Pelot, M. A. and Demorest, K. (2011). Regulation of low-threshold afferent activity may contribute to short-term habituation in *Aplysia californica*. *Neurobiol. Learn. Mem.* **95**, 248-259. doi:10.1016/j.nlm.2010.11.016

Fitts, P. M. and Posner, M. I. (1967). *Human Performance; Basic Concepts in Psychology*, 162 pp. Boston, Massachusetts, USA: Wadsworth.

Gelman, A., Carlin, J. B., Stern, H. S. and Rubin, D. B. (2004). *Bayesian Data Analysis*, 2nd edn., 690 pp. Boca Ratón, FL, USA: Chapman & Hall.

Gerber, B. and Menzel, R. (2000). Contextual modulation of memory consolidation. *Learn. Mem.* **7**, 151-158. doi:10.1101/lm.7.3.151

Giles, A. C. and Rankin, A. H. (2009). Behavioral and genetic characterization of habituation using *Caenorhabditis elegans*. *Neurobiol. Learn. Mem.* **92**, 139-146. doi:10.1016/j.nlm.2008.08.004

Glanzman, D. L. (2011). Olfactory habituation: fresh insights from flies. *Proc. Natl. Acad. Sci. USA* **108**, 14711-14712. doi:10.1073/pnas.1111230108

Groves, P. M. and Thompson, R. F. (1970). Habituation: a dual-process theory. *Psychol. Rev.* **77**, 419-450. doi:10.1037/h0029810

Hemmi, J. M. and Tomsic, D. (2012). The neuroethology of escape in crabs: from sensory ecology to neurons and back. *Curr. Opin. Neurobiol.* **22**, 194-200. doi:10.1016/j.conb.2011.11.012

Hermitte, G., Pedreira, M. E., Tomsic, D. and Maldonado, H. (1999). Context shift and protein synthesis inhibition disrupt long-term habituation after spaced, but not massed, training in the crab *Chasmagnathus*. *Neurobiol. Learn. Mem.* **71**, 34-49. doi:10.1006/nlme.1998.3858

Holmes, S. J. (1911). The reactions of mosquitoes to light in different periods of their life history. *J. Anim. Behav.* **1**, 29-32. doi:10.1037/h0070896

Kaur, J. S., Lai, Y. L. and Giger, A. D. (2003). Learning and memory in the mosquito *Aedes aegypti* shown by conditioning against oviposition deterrence. *Med. Vet. Entomol.* **17**, 457-460. doi:10.1111/j.1365-2915.2003.00455.x

Klein, S. B. (2012). *Learning. Principles and Applications*, 6th edn. Los Angeles, USA: Sage.

Leftwich, A. W. (1954). Vision and blindness in mosquito larvae as indicated by the submerging reflex. *Proc. R. Entomol. Soc. Lond. A* **29**, 156-162. doi:10.1111/j.1365-3032.1954.tb01191.x

Lozada, M., Romano, A. and Maldonado, H. (1990). Long-term habituation to a danger stimulus in the crab *Chasmagnathus granulatus*. *Physiol. Behav.* **47**, 35-41. doi:10.1016/0031-9384(90)90039-7

Lucas, E. A. and Romoser, W. S. (2001). The energetic costs of diving in *Aedes aegypti* and *Aedes albopictus* pupae. *J. Am. Mosq. Control Assoc.* **17**, 56-60.

Magee, L. (1990). R^2 measures based on Wald and likelihood ratio joint significance tests. *Am. Stat.* **44**, 250-253.

Marklewitz, M. and Junglen, S. (2019). Evolutionary and ecological insights into the emergence of arthropod-brone viruses. *Acta Trop.* **190**, 52-58. doi:10.1016/j.actatropica.2018.10.006

McCall, P. J. and Eaton, G. (2001). Olfactory memory in the mosquito *Culex quinquefasciatus*. *Med. Vet. Entomol.* **15**, 197-203. doi:10.1046/j.0269-283x.2001.00304.x

McCall, P. J. and Kelly, D. W. (2002). Learning and memory in disease vectors. *Trends Parasitol.* **18**, 429-433. doi:10.1016/S1471-4922(02)02370-X

McNamara, J. M., Green, R. F. and Olsson, O. (2006). Bayes' theorem and its applications in animal behaviour. *Oikos* **112**, 243-251. doi:10.1111/j.0030-1299.2006.14228.x

Mellamby, K. (1958). The alarm reaction of mosquito larvae. *Entomol. Exp. Appl.* **1**, 153-160. doi:10.1111/j.1570-7458.1958.tb00019.x

Monteith, L. G. (1963). Habituation and associative learning in *Drino bohemica* Mesn. (Diptera: Tachinidae). *Can. Entomol.* **95**, 418-426. doi:10.4039/Ent95418-4

Myers, C. E. and Gluck, M. A. (1994). Context, conditioning, and hippocampal rerepresentation in animal learning. *Behav. Neurosci.* **108**, 835-847. doi:10.1037/0735-7044.108.5.835

Nadel, L. and Willner, J. (1980). Context and conditioning: a place for space. *Physiol. Psichol.* **8**, 218-228. doi:10.3758/BF03332853

Patil, A., Huard, D. and Fonnesbeck, C. J. (2010). PyMC: Bayesian stochastic modelling in Python. *J. Stat. Softw.* **35**, 1. doi:10.18637/jss.v035.i04

Pereyra, P., González Portino, E. and Maldonado, H. (2000). Long-lasting and context-specific freezing preference is acquired after spaced repeated presentations of a danger stimulus in the crab *Chasmagnathus*. *Neurobiol. Learn. Mem.* **74**, 119-134. doi:10.1006/nlme.1999.3945

Pietrantuono, A. L., Enriquez, A., Fernández-Arhex, V. and Bruzzone, O. A. (2015). Substrates preference for pupation on sawfly *Notofenusa surosa* (Hymenoptera: Tenthredinidae). *J. Ins. Behav.* **28**, 257-267. doi:10.1007/s10905-015-9499-z

Pietrantuono, A. L., Bruzzone, O. A. and Fernández-Arhex, V. (2017). The role of leaf cellulose content in determining host plant preferences of three defoliating insects present in the Andean-Patagonian forest. *Aust. Ecol.* **42**, 433-441. doi:10.1111/aec.12460

Pinsker, H., Kupfermann, I., Castellucci, V. and Kandel, E. (1970). Habituation and dishabituation of the GM-Withdrawal reflex in *Aplysia*. *Science* **167**, 1740-1742. doi:10.1126/science.167.3926.1740

Pullen, A. J., Merrill, R. J. N. and Bradshaw, J. W. S. (2012). Habituation and dishabituation during object play in kennel-housed dogs. *Anim. Cogn.* **15**, 1143-1150. doi:10.1007/s10071-012-0538-2

Raderschall, C. A., Magrath, R. D. and Hemmi, J. M. (2011). Habituation under natural conditions: model predators are distinguished by approach direction. *J. Exp. Biol.* **214**, 4209-4216. doi:10.1242/jeb.061614

Rankin, C. H. (2000). Context conditioning in habituation in the nematode *Caenorhabditis elegans*. *Behav. Neurosci.* **114**, 496-505. doi:10.1037/0735-7044.114.3.496

Rankin, C. H. (2009). Introduction to special issue of neurobiology of learning and memory on habituation. *Neurobiol. Learn. Mem.* **92**, 125-126. doi:10.1016/j.nlm.2008.09.010

Rankin, C. H., Beck, C. D. O. and Chiba, C. M. (1990). *Caenorhabditis elegans*: A new model system for the study of learning and memory. *Behav. Brain Res.* **37**, 89-92. doi:10.1016/0166-4328(90)90074-O

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S. et al. (2009). Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. *Neurobiol. Learn. Mem.* **92**, 135-138. doi:10.1016/j.nlm.2008.09.012

Scott, G. (2005). *Essential Animal Behavior*, 1st edn., 216pp. Oxford, UK: Wiley-Blackwell Publishing.

Snoddy, G. S. (1926). Learning and stability: a psychophysiological analysis of a case of motor learning with clinical applications. *J. App. Psychol.* **10**, 1. doi:10.1037/h0075814

Soibam, B., Shah, S., Gunaratne, G. H., Roman, G. W. (2013). Modeling novelty habituation during exploratory activity in *Drosophila*. *Behav. Process.* **97**, 63-75. doi:10.1016/j.beproc.2013.04.005

Thomas, I. M. (1949). The reactions of mosquito larvae to regular repetitions of shadows as stimuli. *Aust. J. Biol. Sci.* **3**, 113-123. doi:10.1071/BI9500113

Thompson, R. F. and Spencer, W. A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.* **73**, 16-43. doi:10.1037/h0022681

Timmermann, S. E. and Briegei, H. (1993). Water depth and larval density affect development and accumulation of reserves in laboratory populations of mosquitoes. *Bull. Soc. Vect. Ecol.* **18**, 174-187.

Tomsic, D., Pedreira, M. E., Romano, A., Hermitte, G. and Maldonado, H. (1998). Context-US association as a determinant of long-term habituation in the crab *Chasmagnathus*. *Anim. Learn. Behav.* **26**, 196-209. doi:10.3758/BF03199212

Tomsic, D., de Astrada, M. B., Sztarker, J. and Maldonado, H. (2009). Behavioral and neuronal attributes of short- and long-term habituation in the crab *Chasmagnathus*. *Neurobiol. Learn. Mem.* **92**, 176-182. doi:10.1016/j.nlm.2009.01.004

Twick, I., Lee, J. A. and Ramaswami, M. (2014). Olfactory habituation in *Drosophila*-odor encoding and its plasticity in the antennal lobe. *Prog. Brain Res.* **208**, 3-38. doi:10.1016/B978-0-444-63350-7.00001-2

Vinauger, C., Lutz, E. K. and Riffell, J. A. (2014). Olfactory learning and memory in the disease vector mosquito *Aedes aegypti*. *J. Exp. Biol.* **217**, 2321-2330. doi:10.1242/jeb.101279

Vinauger, C., Lahondère, C., Cohuet, A., Lazzari, C. R. and Riffell, J. A. (2016). Learning and memory in disease vector insects. *Trends Parasitol.* **32**, 761-771. doi:10.1016/j.pt.2016.06.003

Whitlow, J. W. (1975). Short-term memory in habituation and dishabituation. *J. Exp. Psychol.* **104**, 189-206. doi:10.1037/0097-7403.1.3.189