



Experience-dependent modulation of the attraction to faeces in the kissing bug *Triatoma infestans*



Sofía L. Mengoni, Alicia N. Lorenzo-Figueiras, Sebastián A. Minoli*

DBBE Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IBBEA, CONICET-UBA, CP: 1428 Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 21 March 2016
Received in revised form 25 October 2016
Accepted 26 October 2016
Available online 11 November 2016

Keywords:

Aggregation
Faeces
Learning
Plasticity
Triatoma infestans

ABSTRACT

Triatoma infestans is the main vector of the Chagas disease in Latin America. These nocturnal bugs spend most of the daylight hours aggregated with conspecifics inside crevices in roofs and walls. Around the entrances of the shelters *T. infestans* deposits faeces that contain chemical cues that attract conspecifics. In this work we investigated whether attraction to faeces can be modulated by experience in this insect species. First, we analyzed if the attraction of nymphs to faeces is innate or acquired through previous sensory experiences. Results show that after hatching, 1st instar nymphs are attracted to faeces even if they had never been in contact with them before, thus indicating that this attraction is innate. Second, we studied if attraction to faeces can be influenced by the presence of con-specifics. No differences were found in the attraction to faeces of nymphs released alone or in groups, suggesting that attraction to faeces is independent of the presence of other individuals. Third, we examined if the innate response to faeces of nymphs can be modulated by experience. After pre-exposing nymphs to faeces during 24 h, insects were no longer attracted to faeces. Finally, by pairing the presence of faeces with an aversive mechanical disturbance, nymphs switched from attraction to avoidance of faeces. These results show that although faeces attraction has a strong innate component, it can be modulated by experience. The learning and memory capacities of triatomines have been studied only recently, and our work is the first report on the effects of experience in the aggregation context.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The aggregation of living animals is a widely spread phenomenon observed at various scales and levels of complexity in most biological systems (Hamilton, 1971). The occurrence of this behaviour is mainly governed by individual sensory processes that cause a coincidence in time and space. However, different animals use input from different modalities and diverse external cues to achieve such aggregation. On one hand, an heterogeneous environment clustering favourable conditions can generate a spatial aggregation of the presence of con-specifics. In this way animals share a space just because it is favourable for all of them; e.g. animals assembled around a scarce but rich resource. Alternatively, animals could be attracted to each other independently of environmental conditions, generating a similar pattern, *i.e.* a congregation of individuals. But while in the first case individuals aggregate because they are independently attracted to particular environmental cues, in the second case the attraction causing aggregation is mediated

by cues released by conspecifics. Moreover, both processes might be acting at the same time in natural environments.

Insects are ectothermic animals, as their metabolism is modulated by the external temperature. In some cases, however, insects can control their body temperature by locally heating certain parts of their bodies using their muscles (Heinrich, 1970) or even by assembling with conspecifics in closed areas, a process that can in some cases maintain an internal group temperature and/or humidity different from the external one (Jones and Oldroyd, 2006). Triatomines (Heteroptera: Reduviidae: Triatominae) spend much of their lives aggregated with conspecifics inside their shelters. These nocturnal insects are active at the beginning of the night, when they leave their shelters in search of food, and before dawn as they return to their shelters after feeding (Lazzari, 1992; Lorenzo and Lazzari, 1998). Previous work shows that *Triatoma infestans* deposits faeces around the entrance of their shelters and that the chemical components of these faeces can guide conspecifics to the access of adequate refuges (Lorenzo and Lazzari, 1996). In controlled experimental conditions it was shown that *T. infestans* is attracted to conspecifics faeces (Lorenzo Figueiras et al., 1994; Lorenzo Figueiras and Lazzari, 2000) and even to faeces from different but closely related species (Lorenzo Figueiras and

* Corresponding author.

E-mail addresses: sofi_mengoni@hotmail.com (S.L. Mengoni), aliciaf@bg.fcen.uba.ar (A.N. Lorenzo-Figueiras), minoli@bg.fcen.uba.ar (S.A. Minoli).

Lazzari, 1998, 2002). The faeces aggregation signal is critical for the choice of an adequate shelter in which to remain during the photophase, as remaining outside during daylight hours can expose triatomines to predators. However, many aspects of the attraction to faeces in triatomines are still unknown, some of which we attempt to address in this work.

Although many aspects of the biology and behaviour of triatomines have been well studied, not much information is available about how these behaviours can be modulated by the environment and/or by previous experiences. In nature, countless animals exhibit behaviours which may be more or less rigid depending on the evolutionary history of the species. Many of these behaviours can be modulated by prior sensory experience, that is, experience can produce an increase or a decrease in a particular response related to that stimulus. Moreover, many behavioural responses only occur after a prior sensory experience related to a positive or negative reward, where a neutral stimulus acquires a biological meaning after repeated paired presentations with an innate stimulus. Learning involves a complex series of processes that promote reversible modifications in particular behaviours which can be highly adaptive, generating a memory of that event. Two main types of learning have been well described so far: non-associative and associative. The first one is generated after the repetition of a unique type of stimulus that, without any reinforcement, increases (sensitization) or decreases (habituation) the intensity and/or frequency of the subsequent response of the individual to the same stimulus (Kandel, 1991; Menzel, 1999; Rakitin et al., 1991). Associative learning, in the other hand, occurs after a repeated occurrence of two stimuli (classical conditioning) or an action and a stimulus (operant conditioning) that generates a behavioural output different from the behaviour evoked before the association (Pavlov, 1929; Skinner, 1937). In this type of learning individuals can anticipate events by recognizing marks previously related to them.

The importance of learning and memory in haematophagous insects has been proposed more than 60 years ago, when Nielsen and Nielsen (1953) described that mosquitoes return to places where they had previously fed, a behaviour known as homing behaviour. Other authors also suggested the existence of a spatial memory in mosquitoes as they reported that in natural environments they are capable of learning to associate particular environmental odours with favourable oviposition sites or hosts (Alonso et al., 2003; Bouyer et al., 2007; Chilaka et al., 2012; Kaur et al., 2003; McCall and Eaton, 2001; McCall et al., 2001; McCall and Kelly, 2002; Menda et al., 2012; Mwandawiro et al., 2000; Sanford and Tomberlin, 2011; Service, 1994; Tomberlin et al., 2006; Vinauger et al., 2014). However, Alonso and Schuck-Paim (2006) discuss some of these experimental findings which erroneously ascertain the existence of learning and memory processes in haematophagous insects. More recently, many advances have been made towards understanding the cognitive abilities of haematophagous insects other than mosquitoes. In triatomines, it was shown under controlled conditions that *Rhodnius prolixus* can learn to walk towards or against an originally neutral odour after an appetitive or aversive conditioning, respectively (Vinauger et al., 2011a,b). Moreover, the same authors found that even if *R. prolixus* do not present a preference when odours from a live rat or quail are presented simultaneously, an aversive conditioning generated an avoidance of odours associated with those hosts (Vinauger et al., 2012). Taking advantage of the proboscis extension response elicited by triatomine insects exposed to a warm surface, it was shown that *R. prolixus* is capable of associating previously neutral odours to novel contexts if they are positively rewarded (Vinauger et al., 2013). In a completely different context, it was shown that the escape response of *R. prolixus* to the alarm pheromone can be modulated by both associative and

non-associative conditioning protocols (Minoli et al., 2013). In the feeding context, it has been reported in the same species that ingestion of an appetitive solution can be negatively modulated by a brief pre-exposure to bitter compounds (Pontes et al., 2014). Additionally, it was shown that triatomines' cognitive abilities follow a circadian rhythm (Vinauger and Lazzari, 2015).

In this work we study the learning capabilities of triatomines in the behavioural context of aggregation to chemical signals present in their faeces. Using naïve, recently emerged nymphs, we analyzed if this behaviour is innate or not, and if it can be modulated by previous sensory experiences, including the presence of conspecifics.

2. Materials and methods

2.1. Insects

Experiments were conducted using unfed 1–7 days-old 1st instar nymphs of *T. infestans* reared in our laboratory. Our colony was frequently supplied with insects from the Servicio Nacional de Chagas of Córdoba (Argentina) to minimize endogamy. Recently deposited eggs were weekly collected in clean flasks and kept separated from all other stages in rearing chambers placed in a different room, with controlled temperature (22 ± 2 °C) and photoperiod (12 h light:12 h darkness). Once hatched, unfed 1st instar nymphs do not deposit faeces, which ensures that they were not exposed to faeces volatiles before experiments.

2.2. Experimental design

We set up an experimental device to study the attraction to faeces of insects submitted to different experimental protocols. All the assays were performed in a dark room during the second half of the night of the insects. This experimental temporal window was chosen to roughly match the time at which insects return to their shelters guided by odours from faeces deposited near the entrance of their shelters (Lorenzo Figueiras et al., 1994; Lorenzo and Lazzari, 1998). The experimental device (Fig. 1) consisted of a rectangular acrylic box ($5 \times 2 \times 1$ cm) whose base was covered with clean filter paper. The arena was virtually divided in two equal halves: one of them had a clean piece of filter paper (2×0.5 cm) while the other half contained a paper loaded with faeces. In spatial control series, both papers were kept clean.

In *individual* assays, one 1st instar nymph of *T. infestans* was gently released in the middle of the arena and its behaviour was video-filmed during one hour. In *group* assays, ten 1st instar nymphs were released at once in the centre of the arena and their behaviour was also recorded in video-films during one hour.

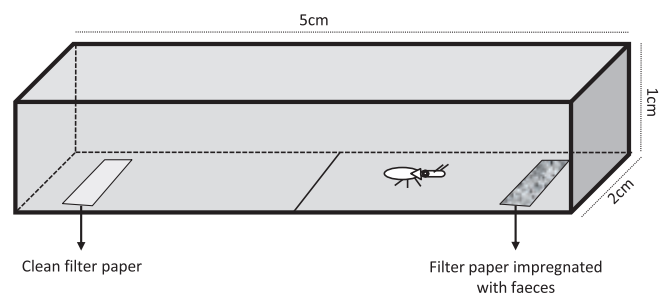


Fig. 1. Experimental device used to investigate the aggregation behaviour of 1st instar nymphs of *T. infestans* evoked by faeces. In *individual* series, one individual was released and the time spent at each side of the arena was expressed as an individual index (IPI). In *group* series, ten individuals were released and the proportion of them found at each side of the arena was expressed as a group index (GPI). In both cases the position of nymphs was registered in videotapes during 1 h.

When stated, before *individual* assays were performed, insects were submitted to different sensory experiences involving faeces, either presented in a non-associative manner (see Experiment 3) or associated with a negative reinforcement (see Experiment 4).

2.3. Collection of faeces

Faeces for the assays were obtained weekly by collecting dejections of recently fed 4th and 5th instar nymphs of *T. infestans* from our colony. This was achieved by placing a clean filter paper covering the base of the alimentary flask containing 40–60 bugs, which were allowed to blood-feed during 45 min over live hens and then left in the same recipient. Then, 3–5 h after feeding ended, the paper with fresh faeces was removed and placed in a clean Petri dish, which was stored separately for 7 days before being used as chemical stimulus during the experiments. Previous reports show that recently deposited faeces are not attractive for *T. infestans*, but they so become 3 h after dejection and up to 10 days after (Lorenzo Figueiras and Lazzari, 2000).

2.4. Parameters and statistical analyses

The insects' behaviour was recorded during one hour in video-films and analyzed using *The Observer 2.01*. To analyse the temporal dynamics of attraction to faeces, we divided the experimental hour in four consecutive 15-min intervals: 0–15, 15–30, 30–45 and 45–60 min.

In *individual* trials we recorded for each 15-min interval the time spent by one insect in each side of the experimental arena and we calculated an individual preference index (*IPI*) every 15 min as follows:

$$IPI_{(time\ interval)} = \frac{T(f) - T(c)}{T(t)}$$

where $T(f)$ is the mean time spent in the side containing faeces, $T(c)$ is the mean time spent in the clean side, and $T(t)$ is the total time. Forty replicates were performed.

To register the spatial preference of insects in *group* trials, we defined 3 static images in each 15-min interval (*i.e.* at times 5, 10 and 15 min since the start of each interval) and we recorded the number of nymphs found at each side of the experimental arena in each one. One only value of spatial preference for each 15-min interval was obtained by averaging the 3 values so obtained. Then we calculated a group preference index (*GPI*) every 15 min as follows:

$$GPI_{(time\ interval)} = \frac{N(f) - N(c)}{N(t)}$$

where $N(f)$ is the mean number of larvae in the side containing faeces, $N(c)$ is the mean number of nymphs in the clean side, and $N(t)$ is the total number of nymphs. Twenty four replicates, each one with 10 nymphs, were performed. Note that the preference index for the group of 10 insects (*GPI*) was calculated using static images as we could not follow the insects' individual position simultaneously during the whole experimental hour.

Both, the *IPI* and the *GPI* could attain values between -1 and 1. Values close to -1, 0 or 1 indicate repulsion, indifference or attraction to faeces, respectively. *One Sample T-Tests* were performed to compare the *IPIs* and *GPIs* corresponding to each time interval against 0 (0 meaning indifference or no spatial preference). *Independent Samples T-Tests* were used to compare the preference of nymphs from *individual* and *group* series. Normality and homoscedasticity of data were tested in all cases.

3. Results

3.1. Experiment 1: innate or acquired attraction to faeces?

Attraction of *T. infestans* to faeces was already addressed by several authors. However, in all cases experiments were performed with nymphs that were previously exposed to faeces from conspecifics. To address if faeces attraction is innate or acquired, we studied the behaviour of faeces-naïve 1st instar nymphs of *T. infestans* (*i.e.* nymphs that had never perceived chemicals present in faeces). These naïve nymphs were individually released in the experimental arena and their attraction to faeces was tested.

Individually released naïve nymphs were not attracted to faeces during the 1st, 2nd and 3rd 15-min intervals (Fig. 2, white triangles; One Sample T-Test, $p > 0.05$ in the three cases). However, nymphs were attracted by faeces during the 4th interval (One Sample T-Test, $p = 0.02$). These results confirm previous reports (Lorenzo Figueiras et al., 1994) showing that nymphs of *T. infestans* aggregate around conspecifics faeces about one hour after being released. Our results further show that attraction to faeces in *T. infestans* is an innate process, and that it requires time (at least 45 min in our experimental device) to be observed.

3.2. Experiment 2: individual vs. group attraction to faeces

The aggregation behaviour of groups of triatomine nymphs towards faeces has been previously studied. Here, in Experiment 1, we reported the individual attraction of larvae to faeces from conspecifics. To investigate if the presence of conspecifics can modulate the individual attraction to faeces, groups of 10 naïve 1st instar nymphs were released in the experimental arena and their attraction to faeces was investigated and compared to that found when the insects were released individually.

Group attraction to faeces was similar to that observed when insects were tested individually (Fig. 2, grey circles). Attraction to faeces was not observed during the 1st, 2nd and 3rd 15-min intervals (One Sample T-Test, $p > 0.05$ in the three cases), but during the 4th interval most insects chose the side of the arena impregnated

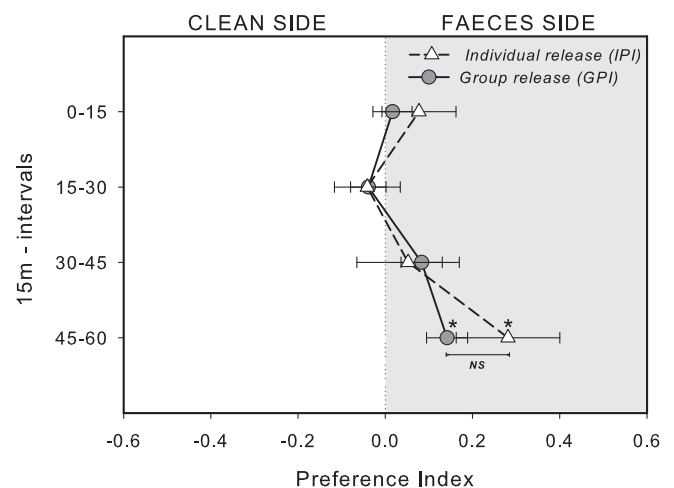


Fig. 2. Temporal dynamics of attraction to faeces of naïve 1st instar nymphs of *T. infestans*. Both, when released individually or in groups, naïve nymphs presented a similar temporal pattern: attraction to faeces only after 45 min. The Preference Index (mean \pm SE) expresses the attraction to faeces as the time spent by an individual (*IPI*) or the number of bugs found (*GPI*) at each side of the arena: 0 = no preference, -1 = repulsion and 1 = attraction. Asterisks denote significant differences against 0 (One Sample T-Test, $p < 0.05$). "NS" expresses no significant differences between attraction to faeces of different series (Independent Samples T-Test, $p > 0.05$).

with faeces (One Sample T-Test, $p = 0.003$). The aggregation levels of *individually* and *group* released insects during the 4th 15-min interval did not differ statistically (Two Sample T-Test, $p > 0.05$), suggesting that the attraction to faeces in *T. infestans* nymphs is independent of the presence of conspecifics.

3.3. Experiment 3: non-associative pre-exposure to faeces

In experiment 1 we showed that nymphs of *T. infestans* are innately attracted to faeces. Here, we determined if this innate response can be modulated by a previous non-associative sensory experience. To this end, 1st instar nymphs were placed for 24 h in a circular plastic flask (2 cm diam., 2 cm height) whose floor was covered with a filter paper impregnated with faeces. Control insects were maintained for 24 h in a similar plastic flask but with a clean filter paper on the floor. Two minutes after the end of pre-exposure, their attraction behaviour was tested in *individual* assays.

Control insects showed a temporal rejection to faeces during the 1st 15-min interval (Fig. 3, white triangles; One Sample T-Test, $p = 0.03$). Subsequently, during the 2nd and 3rd intervals they showed no preference to faeces (One Sample T-Test, $p > 0.05$ in both cases) but in the 4th 15-min interval, they showed the expected preference for the side containing faeces (One Sample T-Test, $p = 0.04$). In contrast, insects that were pre-exposed to faeces (Fig. 3, grey circles) did not show a preference for the side of the arena with faeces during the entire experimental hour (One sample T-Test, $p > 0.05$ in the four cases). These results suggest that a long pre-exposure to faeces can modulate the insect's innate attraction to faeces. Our results, however, cannot address whether this behavioural change results from habituation, peripheral sensory adaptation, or other processes.

3.4. Experiment 4: associative aversive conditioning

To determine if the innate attraction to faeces can be reversed through an associative aversive learning, 1st instar nymphs were submitted to a single paired presentation of faeces and a disturbing mechanical vibration. Nymphs were placed in a circular plastic flask (2 cm diam., 2 cm height) with a filter paper impregnated

with faeces as a substrate. The flask was attached to a laboratory mixer (ZX3 Vortex mixer 115V/60 Hz) set to 40 rpm which vibrated the insect's substrate. We conducted a single training trial in which the mechanical vibration was paired with faeces during 1 min. Control insects received the same treatment, but in a flask with a clean filter paper. Two minutes after the end of the faeces-vibration contingency, the attraction of insects to faeces was tested in *individual* assays.

Control insects did not prefer the side of the arena presenting the faeces along the whole experimental hour (Fig. 4, white triangles; One Sample T-Test, $p > 0.05$ in the four cases). In contrast, insects trained with the pairing of faeces and mechanical vibration (Fig. 4, grey circles) showed no faeces preference during the 1st and 2nd 15-min intervals (One Sample T-Test, $p > 0.05$ in both cases), but avoided the side of the arena with faeces during the 3rd and 4th 15-min intervals (One Sample T-Test, $p = 0.0003$ and $p = 0.0009$, respectively). These results suggest that the insects were able to associate the presence of faeces with a negative reinforcement, and in consequence reversed their original attraction to them. This reversion evinces that the negative value of the mechanical vibration was subjectively higher than the positive attraction exerted by the faeces *per se*.

4. Discussion

Behavioural plasticity is a key process governing the survival of species in unpredictable environments, as it allows animals to adjust their behaviour in response to the resource availability. Any particular action of an individual as a response to an external cue could be innately loaded in its genetics, *i.e.* an instinctive unconditioned innate response. However, in many cases experience can modulate a particular response by increasing or decreasing the intensity of such behavioural outcome, or even generating a new conditioned action in response to a stimulus that did not evoke an innate behaviour. Here we show that under a particular context, that is, the attraction towards compounds present in con-specifics faeces, triatomine insects can modify their behaviour according to their previous experience.

In the first experimental series of this work (Experiment 1) we analyzed if the attraction of *T. infestans* to faeces is innately

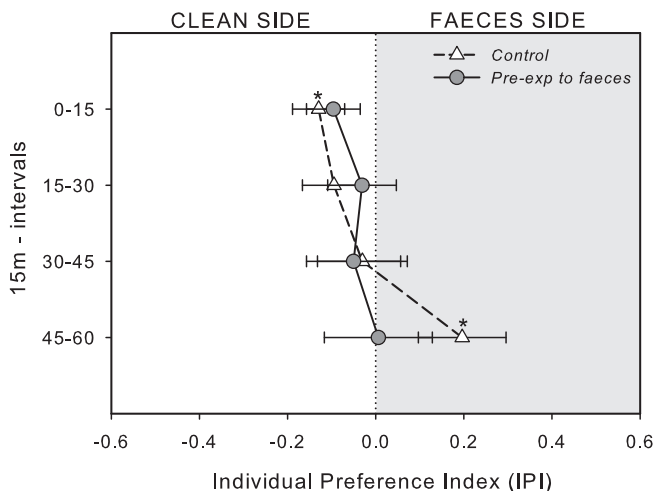


Fig. 3. Temporal dynamics of attraction to faeces of naive and pre-exposed 1st instar nymphs of *T. infestans*. Naive nymphs (control) preferred the faeces side after 45 min. This preference disappeared after a 24 h pre-exposure to faeces. The Individual Preference Index (IPI, mean \pm SE) expresses the attraction to faeces as the time spent by an individual at each side of the arena: 0 = no preference, -1 = repulsion and 1 = attraction. Asterisks denote significant differences against 0 (One Sample T-Test, $p < 0.05$).

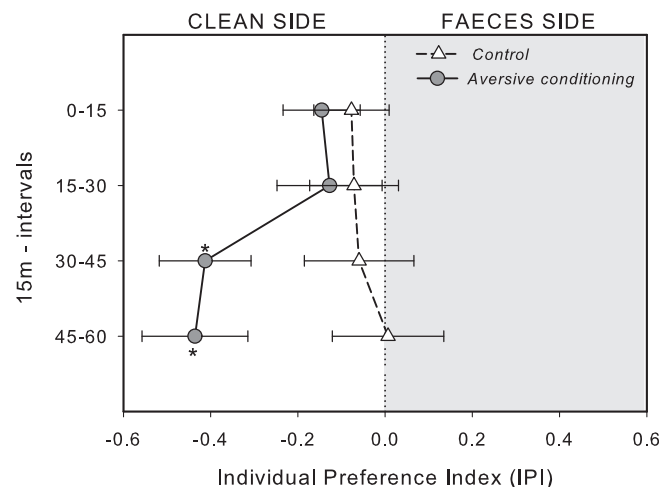


Fig. 4. Temporal dynamics of attraction to faeces 1st instar nymphs of *T. infestans* submitted to an aversive conditioning. Nymphs exhibited a clear switch from attraction to repulsion to faeces after associating the presence of faeces with a negative reinforcement (mechanical disturbance). The Individual Preference Index (IPI, mean \pm SE) expresses the attraction to faeces as the time spent by an individual at each side of the arena: 0 = no preference, -1 = repulsion and 1 = attraction. Asterisks denote significant differences against 0 (One Sample T-Test, $p < 0.05$).

expressed or if it is acquired after several rewarded or not experiences. Previous reports have shown that triatomines are attracted to faeces (Lorenzo Figueiras et al., 1994; Lorenzo and Lazzari, 1996; Lorenzo Figueiras and Lazzari, 1998; Lorenzo Figueiras and Lazzari, 2000, 2002; Reisenman et al., 2000; Pires et al., 2002; Vitta et al., 2002). However, as nymphs used in these reports were obtained from general rearing chambers where all instar live together (*i.e.* sharing a chemical ambient that included volatiles released by conspecifics' faeces), whether this attraction was innate or not, or if it could be modulated by previous sensory experiences could not be addressed. Our results show that triatomines are innately attracted to faeces, evincing for the first time that the olfactory system of recently emerged nymphs is readily functional after emergence. Up to date and to our knowledge, this is the first report in which the innate response to a chemical cue is analyzed in detail in a haematophagous insect. Still, the occurrence of innate behaviours in response to other chemical cues or to cues of different sensory modality remains to be investigated.

The assembly of nymphs over or around a paper impregnated with dry faeces can result from different processes acting independently or simultaneously. For example, it can be the consequence of numerous individuals attracted independently to faeces, but it can also be triggered by an aggregation process in which nymphs are attracted to each other in the presence of faeces. Also, these two processes can occur simultaneously resulting in insect aggregates. Both types of processes have been already reported in triatomines. When released in groups, bugs trend to aggregate over or near a faeces-impregnated piece of paper deposited at one side of a circular experimental arena (Lorenzo Figueiras et al., 1994), but it has also been reported that insects aggregate even if there are no external cues added to the arena (Minoli et al., 2007). Moreover, the aggregation in absence of faeces occurred rhythmically: triatomines were more assembled during daylight hours than during scotophase (Minoli et al., 2007). This suggests that, at least in such experiments, an attraction to cues released by conspecifics is the main force ruling the aggregation. We show here for the first time that nymphs of *T. infestans* evince the same degree of attraction/aggregation over the faeces when released individually or in groups (Experiment 2). Thus, an individual attraction to faeces is probably the process guiding the assembling of bugs around faeces. Our results suggest that in the case of triatomines that are found aggregated in natural shelters, insects are individually attracted by faeces deposited around its entrance, and once inside, other aggregation factors might act to arrest them inside the refuges.

In the third experimental series (Experiment 3) we analyzed whether the innate attraction to faeces can be modulated by experience, by pre-exposing insects to faeces before tests. During the 1st 15-min interval (Fig. 3) control larvae (*i.e.* not pre-exposed to faeces) showed a temporal rejection to faeces. This result was probably the consequence of a secondary effect caused by the manipulation of the insects before tests. Note that a similar result was observed for the group pre-exposed to faeces and for the groups that were submitted to the associative aversive conditioning during the 1st 15-min interval (Experiment 4, see Fig. 4).

We found that a 24 h pre-exposure to faeces can generate an extinction of the innate attraction to faeces, particularly evinced in the 4th 15-min interval (Fig. 3). This is the first time that a behavioural plasticity under an aggregation context is evinced. This result is can become highly relevant for epidemiological purposes, as triatomines use their olfactory sense to locate suitable shelters before dusk. If we were able to inhibit the faeces-related olfactory pathway at longer time scales (*i.e.* not just a few minutes after treatment), it would be more difficult for them to find a protected refuge to spend daylight hours. However, results presented here do not allow determining if the lack of response to a previously attractive cue is mainly due to a peripheral sensory adaptation, if a cen-

tral habituation process can be responsible for this extinction, or if both processes can be acting together. Generally, reversal of sensory adaptation occurs at shorter times (*e.g.* a couple of seconds) as compared to habituation processes that can last for several minutes. As the time between the end of pre-exposure and the test was never less than 2 min, we suggest that an habituation process is underlying the decrease in attraction. Even if habituation was originally described as a decrease in a response after a repeated exposure to a stimulus (see review by Rankin et al., 2009), the habituation of a response to a stimulus after a single continuous exposure to the same stimulus has been largely reported (*e.g.* Chaput and Panhuber, 1982; Sachin and Upinder, 2003; Wilson, 1988). Further experiments are needed to ascertain this suggestion.

Surprisingly, by applying an associative aversive paradigm (Experiment 4) we were able to switch the nymphs' behaviour from faeces attraction to repulsion. Thus, these results allow us to propose that the associative-dependent modulation of the attraction to faeces in triatomines can be quite radical, since nymphs could rapidly switch from being attracted to being repelled by a particular chemical signal. Moreover, the intensity of the avoidance ($IPI \cong -0.45$) was comparatively higher than the attraction ($IPI \cong 0.25$) to faeces. This fact can only be achieved if the negative perceptual value of the reinforcement (*i.e.* the mechanical vibration) is somehow stronger than the potentially advantage of remaining over or near the attractive cue released by faeces. Unexpectedly, larvae from the control group, in which animals received the mechanical punishment without perceiving faeces, were not attracted to faeces during the 4th 15-min interval. This result suggests that the mechanical vibration alone can generate a modification in the attraction behaviour of triatomines, probably due to a disruption effect generated by a disturbing event. However, only the paired presentation of faeces and the negative reinforcement triggered the switch from attraction to repulsion, suggesting that an associative process is involved in such experience-dependent modulation.

These results evince that haematophagous bugs are capable of learning from their experiences, and thus modify their behaviour. In natural environments *T. infestans* deposits their faeces mainly near the entrance of their shelters and use this chemical information to find an adequate shelter. However, some shelters can change from suitable to non-suitable, for what the behavioural plasticity of the faeces-mediated attraction can become relevant to subsequently avoid shelters that resulted aversive in a first experience. With our work, we added knowledge about the learning abilities of non-classical biological models for learning and memory (*i.e.* different than bees, flies and mice, among other), which can attain great relevance in a comparative approach. Moreover, because triatomines are haematophagous insects of epidemiological relevance to humans, results presented here could be used to increase the efficiency of control and/or monitoring programs for controlling Chagas disease transmission.

Acknowledgements

This work was supported by AGENCIA NACIONAL DE PROMOCION CIENTIFICA Y TECNOLOGICA (ANPCyT), Argentina, PICT 2013-1253. Special thanks to Martin Giurfa for helpful comments on the manuscript. We also thank the anonymous referees for valuable observations.

References

- Alonso, W.J., Schuck-Paim, C., 2006. The ghosts that pester studies on learning in mosquitoes: guidelines to chase them off. *Med. Vet. Entomol.* 20, 157–165.

- Alonso, W.J., Wyatt, T.D., Kelly, D.W., 2003. Are vectors able to learn about their hosts? A case study with *Aedes aegypti* mosquitoes. *Mem. Inst. Oswaldo Cruz* 98, 665–672.
- Bouyer, J., Pruvot, M., Bengaly, Z., Guerin, P.M., Lancelot, R., 2007. Learning influences host choice in tsetse. *Biol. Lett.* 3, 113–117.
- Chaput, M.A., Panhuber, H., 1982. Effects of long duration odor exposure on the unit activity of olfactory bulb cells in awake rabbits. *Brain Res.* 250, 41–52.
- Chilaka, N., Perkins, E., Tripet, F.D.R., 2012. Visual and olfactory associative learning in the malaria vector *Anopheles gambiae* sensu stricto. *Malaria J.* 11, 1–11.
- Hamilton, W.D., 1971. Geometry for the selfish herd. *J. Theor. Biol.* 31, 295–311.
- Heinrich, B., 1970. Nervous control of the heart during thoracic temperature regulation in a Sphinx moth. *Science* 169, 606–607.
- Jones, J.C., Oldroyd, B.P., 2006. Nest thermoregulation in social insects. *Adv. Insect Physiol.* 33, 153–191.
- Kandel, E.R., 1991. Cellular mechanisms of learning and the biological basis of individuality. In: Kandel, E.R., Schwartz, J., Jessel, T.M. (Eds.), *Principles of Neural Sciences*. third ed. Appleton and Lange, East Norwalk, pp. 1010–1030.
- Kaur, J.S., Lai, Y.L., Giger, A.D., 2003. Learning and memory in the mosquito *Aedes aegypti* shown by conditioning against oviposition deterrence. *Med. Vet. Entomol.* 17, 457–460.
- Lazzari, C.R., 1992. Circadian organization of locomotion activity in the haematophagous bug *Triatoma infestans*. *J. Insect Physiol.* 38, 895–903.
- Lorenzo, M.G., Lazzari, C.R., 1996. The spatial pattern of defecation in *Triatoma infestans* and the role of faeces as a chemical mark of the refuge. *J. Insect Physiol.* 42, 903–907.
- Lorenzo, M.G., Lazzari, C.R., 1998. Activity pattern in relation to refuge exploitation and feeding in *Triatoma infestans* (Hemiptera: Reduviidae). *Acta Trop.* 70, 163–170.
- Lorenzo Figueiras, A.N., Lazzari, C.R., 1998. Aggregation behaviour and interspecific responses in three species of Triatominae. *Mem. Inst. Oswaldo Cruz* 93, 133–137.
- Lorenzo Figueiras, A.N., Lazzari, C.R., 2000. Temporal change of the aggregation response in *Triatoma infestans*. *Mem. Inst. Oswaldo Cruz* 95, 889–892.
- Lorenzo Figueiras, A.N., Lazzari, C.R., 2002. Aggregation behaviour and interspecific responses in *Rhodnius prolixus* Stal. *Mem. Inst. Oswaldo Cruz* 97, 569–571.
- Lorenzo Figueiras, A.N., Kenigsten, A., Lazzari, C.R., 1994. Aggregation in the haematophagous bug *Triatoma infestans*: chemical signals and temporal pattern. *J. Insect Physiol.* 40, 311–316.
- McCall, P.J., Eaton, G., 2001. Olfactory memory in the mosquito *Culex quinquefasciatus*. *Med. Vet. Entomol.* 15, 197–203.
- McCall, P.J., Kelly, D.W., 2002. Learning and memory in disease vectors. *Trends Parasitol.* 18, 429–433.
- McCall, P.J., Moshia, F.W., Njunwa, K.J., Sherlock, K., 2001. Evidence for memorized site-fidelity in *Anopheles arabiensis*. *Trans. R. Soc. Trop. Med. Hyg.* 95, 587–590.
- Menda, G., Uhr, J.H., Wyttenbach, R.A., Vermeylen, F.M., Smith, D.M., Harrington, L. C., Hoy, R.R., 2012. Associative learning in the dengue vector mosquito, *Aedes aegypti*: avoidance of a previously attractive odor or surface color that is paired with an aversive stimulus. *J. Exp. Biol.* 216, 218–223.
- Menzel, R., 1999. Memory dynamics in the honeybee. *J. Comp. Physiol. A* 185, 323–340.
- Minoli, S., Baraballe, S., Lorenzo Figueiras, A., 2007. Daily rhythm of aggregation in the haematophagous bug *Triatoma infestans* (Heteroptera: Reduviidae). *Mem. Inst. Oswaldo Cruz* 102, 449–454.
- Minoli, S., Palottini, F., Manrique, G., 2013. The main component of an alarm pheromone of kissing bugs plays multiple roles in the cognitive modulation of the escape response. *Front. Behav. Neurosci.* 7, 77.
- Mwandawiro, C., Boots, M., Tuno, N., Suwonkerd, W., Tsuda, Y., Takagi, M., 2000. Heterogeneity in the host preference of Japanese encephalitis vectors in Chiang Mai, northern Thailand. *Trans. R. Soc. Trop. Med. Hyg.* 94, 238–242.
- Nielsen, E.T., Nielsen, A.T., 1953. Field observations on the habits of *Aedes taeniorhynchus*. *Ecology* 34, 141–156.
- Pavlov, I.P., 1929. *Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex*. Ed: Oxford University Press, Humphrey Milford, London.
- Pires, H.H., Lorenzo, M.G., Diotaiuti, L., Lazzari, C.R., Lorenzo Figueiras, A.N., 2002. Aggregation behaviour in *Panstrongylus megistus* and *Triatoma infestans*: inter and intraspecific responses. *Acta Trop.* 81, 47–52.
- Pontes, G., Minoli, S., Ortega Insaurralde, I., de Brito Sanchez, M.G., Barrozo, R.B., 2014. Bitter stimuli modulates the feeding decision of a blood-sucking insect via two sensory inputs. *J. Exp. Biol.* 217, 3708–3717.
- Rakitin, A., Tomsic, D., Maldonado, H., 1991. Habituation and sensitization to an electrical shock in the crab *Chasmagnathus*. Effect of background illumination. *Physiol. Behav.* 50, 477–487.
- Rankin, C.H., Abrams, T., Barry, R., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., McSweeney, F.K., Wilson, D.A., Wu, C.F., Thompson, R.F., 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol. Learn. Mem.* 92 (2), 135–138.
- Reisenman, C.E., Lorenzo Figueiras, A.N., Giurfa, M., Lazzari, C.R., 2000. Interaction of visual and olfactory cues in the aggregation behaviour of the haematophagous bug *Triatoma infestans*. *J. Comp. Physiol. A* 186, 961–968.
- Sachin, S.D., Upinder, S.D., 2003. Representation of odor habituation and timing in the hippocampus. *J. Neurosci.* 23 (5), 1903–1915.
- Sanford, M.R., Tomberlin, J.K., 2011. Conditioning individual mosquitoes to an odor: sex, source, and time. *PLoS One* 6, e24218.
- Service, M.W., 1994. Male swarming of the mosquito *Culex (Culex) torrentium* in England. *Med. Vet. Entomol.* 8, 95–98.
- Skinner, B.F., 1937. Two types of conditioned reflex: a reply to Konorski and Miller. *J. Genet. Psychol.* 16, 272–279.
- Tomberlin, J.K., Rains, G.C., Allan, S.A., Sanford, M.R., Lewis, W.J., 2006. Associative learning of odor with food- or blood-meal by *Culex quinquefasciatus* Say (Diptera: Culicidae). *Naturwissenschaften* 93, 551–556.
- Vinauger, C., Lazzari, C.R., 2015. Circadian modulation of learning ability in a disease vector insect, *Rhodnius prolixus*. *J. Exp. Biol.* 218, 3110–3117.
- Vinauger, C., Buratti, L., Lazzari, C.R., 2011a. Learning the way to blood: first evidence of dual olfactory conditioning in a blood-sucking insect, *Rhodnius prolixus*. I. Appetitive learning. *J. Experiment. Biol.* 214, 3032–3038.
- Vinauger, C., Buratti, L., Lazzari, C.R., 2011b. Learning the way to blood: first evidence of dual olfactory conditioning in a blood-sucking insect, *Rhodnius prolixus*. II. Aversive learning. *J. Experiment. Biol.* 214, 3039–3045.
- Vinauger, C., Pereira, M.H., Lazzari, C.R., 2012. Learned host preference in a Chagas disease vector, *Rhodnius prolixus*. *Acta Trop.* 122, 24–28.
- Vinauger, C., Lallement, H., Lazzari, C.R., 2013. Learning and memory in *Rhodnius prolixus*: habituation and aversive operant conditioning of the proboscis extension response. *J. Experiment. Biol.* 216, 892–900.
- Vinauger, C., Lutz, E.K., Riffell, J.A., 2014. Olfactory learning and memory in the disease vector mosquito *Aedes aegypti*. *J. Experiment. Biol.* 217, 2321–2330.
- Vitta, A.C., Lorenzo Figueiras, A.N., Lazzari, C.R., Diotaiuti, L., Lorenzo, M.G., 2002. Aggregation mediated by faeces and footprints in *Triatoma pseudomaculata* (Heteroptera: Reduviidae), a Chagas disease vector. *Mem. Inst. Oswaldo Cruz* 97, 865–867.
- Wilson, D.A., 1988. Habituation of odor responses in the rat anterior piriform cortex. *J. Neurophysiol.* 79, 1425–1440.