Behavioral asymmetries in ticks – Lateralized questing of Ixodes ricinus to a mechatronic apparatus delivering host-borne cues

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ABSTRACT

Ticks are considered among the most dangerous arthropod vectors of disease agents to both humans and animals worldwide. Lateralization contributes to biological fitness in many animals, conferring important functional advantages, therefore studying its role in tick perception would critically improve our knowledge about their host-seeking behavior. In this research, we evaluated if Ixodes ricinus (L.) (Ixodiidae) ticks have a preference in using the right or the left foreleg to climb on a host. We developed a mechatronic device moving a tuft of fox skin with fur as host-mimicking combination of cues. This engineered approach allows to display a realistic combination of both visual and olfactory host-borne stimuli, which is prolonged over the time and standardized for each replicate. In the first experiment, the mechatronic apparatus delivered host-borne cues frontally, to evaluate the leg preference during questing as response to a symmetrical stimulus. In the second experiment, hostborne cues were provided laterally, in an equal proportion to the left and to the right of the tick, to investigate if the host direction affected the questing behavior. In both experiments, the large majority of the tested ticks showed individual-level left-biased questing acts, if compared to the ticks showing right-biased ones. Furthermore, population-level left-biased questing responses were observed post-exposure to host-mimicking cues provided frontally or laterally to the tick. Overall, this is the first report on behavioral asymmetries in ticks of medical and veterinary importance. Moreover, the mechatronic apparatus developed in this research can be exploited to evaluate the impact of repellents on tick questing in highly reproducible standardized conditions.

1. Introduction

Ticks are considered among the most dangerous arthropod vectors of disease agents to both humans and animals worldwide (Colwell et al., 2011; Jongejan and Uilenberg, 2004), holding a vector competence surpassed only by mosquitoes (Sonenshine et al., 2002; Bissinger and Roe, 2010; Benelli et al., 2017a). Good examples of diseases vectored by ticks include Lyme's disease, caused by Borrelia spp. bacteria, Rocky Mountain spotted fever, anaplasmosis, ehrlichiosis, Powassan virus, and piroplasmosis (Estrada-Peñã and Jongejan, 1999; Dantas-Torres et al., 2012; Ebani et al., 2017). Ticks include approximately 900 species, which are distributed in the two main families Argasidae and Ixodidae, and the Nutalliellidae family, including only a species (Nuttalliella namaqua, Bredford) (Pfäffle et al., 2013; Guglielmone et al., 2014).

Ixodidae, commonly known as hard ticks, are responsible for most of the cases of human parasitization, although also Argasidae, commonly known as soft ticks, sporadically bite humans (Dantas-Torres and Otranto, 2016). Human parasitization is caused by several tick species that are different from region to region and generally infest animals representing a reservoir host of several pathogen micro-organisms (Piesman and Eisen, 2008).

Both biotic (e.g., host density) and abiotic (e.g., weather) factors can play an important role in affecting the host-seeking activity in ticks, which is directly correlated with the host risk of contracting tick-borne diseases (Loye and Lane, 1988; Schulze et al., 2001; Hubálek et al., 2003). However, ticks cognitive endogenous factors are also worth to be investigated in the host seeking/contacting behavior. During host seeking, hard ticks (Ixodidae) exhibit a display called questing as a way of increasing the chances of coming in to contact with a suitable mammal host. The behavior involves the tick climbing up a blade of grass or other similar plant parts and then waiting with its forelegs outstretched. As a host passes by it brushes against the forelegs of the tick, which bear Haller's organ and the tick grabs hold of the host (Lees, 1948; Randolph and Storey, 1999; Perret et al., 2000).

To our mind, tick questing is an ideal display to investigate behavioral asymmetries. Nowadays, it is widely recognized that lateralization has a key role for the development of several cognitive functions in vertebrates and invertebrates as well (Vallortigara et al., 1999, 2011; Bisazza et al., 2000; Vallortigara, 2000, 2006; Rogers, 1989, 2000, 2002, 2017; Vallortigara and Rogers, 2005a,b; Rogers et al., 2013a,b; Kelley et al., 2017). Regarding arthropods, studies on behavioral asymmetries mostly focused on insects and crustaceans (see Frasnelli et al., 2012a and Frasnelli, 2013 for dedicated reviews), while limited information is available on spiders (Heuts and Lambrechts, 1999; Ades and Ramires, 2002; Ruhland et al., 2017). However – to the best of our knowledge – no findings are available concerning lateralization and behavioral asymmetries in tick vectors of public health importance, and more generally in the subclass of Acarina.

Lateralization contributes significantly to biological fitness in many animals, conferring important functional advantages (Rogers and Andrew, 2002; Frasnelli, 2013; Versace and Vallortigara, 2015), therefore studying its role in tick perception would critically improve our knowledge about their host-seeking behavior. Since Ixodidae climb on top of the vegetation assuming a questing posture to intercept the host (Norval et al., 1987; Randolph and Storey, 1999; Perret et al., 2000), we investigated if ticks have any lateral bias in the use of the forelegs when a host was presented to a questing tick.

To evaluate if Ixodes ricinus (L.) (Ixodiidae) ticks have a preference in using the right or the left foreleg to climb on a host, here a mechatronic device that moved a tuft of fox skin with fur as host-mimicking combination of cues was developed. This engineered approach allows to display a realistic combination of host-borne cues (i.e., both visual and olfactory stimuli) that is prolonged over the time and standardized for each replicate (Todd, 1993; Partan, 2004; Krause et al., 2011; Romano et al., 2017b,c). The mechatronic apparatus delivered hostborne cues frontally to the I. ixodes tick, to evaluate the leg preference during questing as response to a symmetrical stimulus. Furthermore, we provided the host-borne cues laterally, in an equal proportion to the left and to the right of the I. ricinus tick, to investigate if the host direction affects the tick perception, thus questing behavior.

2. Materials and methods

2.1. Ethical note

This research adheres to the guidelines for the treatment of animals in behavioral research and teaching (ASAB/ABS, 2014), the laws of the country (Italy) in which the study was performed (D.M. 116192) and the European Union regulations (European Commission, 2007).

2.2. Ticks and general observations

Ixodes ricinus adult females were obtained from fresh carcasses of
Vulpes vulpes L. of both genders shot during the regular hunting season in the Province of Pisa (43° N, 10–11° E) (Ebani et al., 2017). I. ricinus individuals were collected immediately after the death of foxes, since they spontaneously drop off the hosts. Ticks were identified relying to the systematic keys by Estrada-Penã et al. (2004), then stored in clean glass vials (diameter 10 mm, length 50 mm) for 6 h, until the testing phase. Each tick was provided with a wet filter paper disc (diameter 10 mm) dipped in tap water. Damaged ticks were discarded and not used for behavioral experiments. Engorged individuals were not selected for the experiments because of their reduced mobility and responsiveness. Fresh tufts of fox skin with fur used to trigger tick questing through the mechatronic apparatus were obtained from the V. vulpes carcasses mentioned above.

Experiment were conducted in March 2017. All observations were carried out in a Petri dish arena (diameter 100 mm; height: 10 mm) from 11:00 to 19:00 h, at 25 °C and 65% RH. The arena was carefully washed after each replicate following the method by Carpita et al. (2012). The room was illuminated with fluorescent daylight tubes [16:8 (L:D) photoperiod, lights on at 6:00]. Neon tubes (Philips 30 W/33) were used, and the light intensity in close proximity of the testing arena
was 1000 lx, estimated over the 300- to 1100-nm waveband using a LI-1800 spectroradiometer (LI-COR, Lincoln, NE, USA), equipped with a remote cosine receptor (Benelli et al., 2017b). All trials were focally recorded by an observer. To avoid visual cues from the observer affecting the behavior of the tested ticks, a white wall of filter paper (Whatman no.1, height 30 cm) surrounded the experimental arena (Romano et al., 2016a).

2.3. Mechatronic apparatus delivering host-borne cues

Here, the host-seeking behavior of I. ricinus ticks were observed after transferring a tick into the testing arena and exposing the individual to a mechatronic apparatus (Fig. 1) presenting the host-mimicking
cues in close proximity of the tick. The mechatronic apparatus delivering host-borne cues was composed by a servomotor (Hard HS 3004) connected to a rotor (diameter 50 mm) in acrylonitrile butadiene styrene (ABS), designed in SolidWorks and fabricated by rapid prototyping.

As a host-mimicking combination of visual and olfactory stimuli, a tuft of fox skin with fur (length: 60 mm; width: 30 mm), tied to a hole close to the circumference of the rotor, was placed and moved by controlling the servomotor with a microcontroller (Arduino, Mega 2560). This simple system was located on a suspended platform upon the test arena, to move the fox fur close to the tested tick (Fig. 1).

2.4. Lateralized questing experiments

In the first experiment, we used the mechatronic apparatus described above to present the combination of host-mimicking cues to the I. ricinus tick frontally. Once the tick extended the forelegs exhibiting the "questing posture" (Lees, 1948), the robotic combination of cues was frontally brought about 5 mm from the tick palps and the leg used to transfer itself on the host-mimicking cues was noted. Thirty replicates were performed for each tick. In the second experiment, a tick was placed in the testing arena and the combination of host-borne cues was swung perpendicularly to the longitudinal body axis of the tick, 5 mm from its palps, after the tick questing posture occurred.

In both experiments, for each tested tick, we recorded (i) the number of climbing attempts occurred when the cues were provided from the frontal or lateral side of the tick, (ii) the first leg used to attempt climbing, as well as (iii) the climbing success (e.g., when a tick intercepted the delivered combination of cues and fixed itself on). Furthermore, the duration of tick anchoring on the stimulus was recorded, evaluating the number of ticks fixed on the host's fur for more than 30 s. Ticks that were not involved in any seeking behavior or that were constrained to the side of the arena were discarded. Each tick was tested thirty times.

2.5. Statistical analysis

Concerning I. ricinus lateralized questing data, population-level differences in the overall number of ticks displaying a lateralized response was analyzed using a likelihood ratio χ_2 test with Yates' correction (Sokal and Rohlf, 1981). Results were considered statistically significant using P= 0.05 as threshold. Individual-level lateralization in the tested ticks was computed calculating the laterality index (LI) following the formula by Frasnelli et al. (2012b): LI= (R − L/R + L). Where R and L indicate, respectively, the normalized mean number of times in which each tick use the right or the left anterior leg during questing; a score of 1.0 indicated exclusive use of the right leg, while a score of −1.0 indicated exclusive use of the left leg. A score of 0 indicated equal numbers of right and left legs during questing acts. Ticks with LI ranging from −0.2 to 0.2 were not considered as lateralized.

3. Results and discussion

The lateralized questing behavior in I. ricinus ticks exposed frontally to a mechatronic apparatus delivering a combination of host-mimicking cues was described in Table 1, showing individual-level lateralization, with left-biased questing displays (16 on a total of 25 ticks, each tested 30 times). Furthermore, left-biased LI (22 on a total of 25 ticks, each tested 30 times) were observed when the combination of host-mimicking stimuli was presented to the ticks from a lateral side (Table 2).

In both experiments, it was observed that the large majority of the tested I. ricinus ticks exhibited left-biased questing acts, if compared to the ones showing right-biased LI values (Tables 1 and 2).

Population-level questing responses of I. ricinus ticks were shown in Fig. 2. We noted significant left-biased questing in I. ricinus ticks postexposure to a combination of host-mimicking cues provided frontally (χ_2 =4.208; d.f. = 1; P < 0.05) as well as from the left or the right side (χ_2= 15.423; d.f. = 1; P < 0.05) of the tick.

During our literature survey searching for behavioral asymmetries in ticks and other mites, we faced a severe lack of knowledge. No researches on the topics have been found for in whole subclass Acarina. More generally, only three studies are available for the Arachnida class (Heuts and Lambrechts, 1999; Ades and Ramires, 2002; Ruhland et al., 2017). Motor right-left asymmetries have been indirectly evidenced studying eighteen families of spiders, where a small but significant majority (55%) of field captured spiders mainly showed left-leg lesions (Heuts and Lambrechts, 1999), arguing that the left-side vulnerability can be linked to both anatomical (e.g., joint strength and leg length) and behavioral factors (e.g., propensity to move the legs on one side or side-biases present in spiders' predators) as earlier documented in several vertebrate species (Vallortigara and Rogers, 2005a,b). Later, Ades and Ramires (2002) showed that spitting spiders Scytodes globula Nicolet (Araneae) use more frequently their left anterior legs over the right ones to handle their preys. Very recently, it has been highlighted that male tarantula spider (Brachypelma albopilosum Valerio) are rightbiased when choosing in a T-maze between two identical cues (e.g., light or female odors) (Ruhland et al., 2017).

On the other hand, concerning insects, many research evidences are available on lateralized traits in social and solitary bees (e.g., Anfora et al., 2010, 2011; Frasnelli et al., 2010a,b, 2011; Rigosi et al., 2011; Rogers and Frasnelli, 2016; Rogers et al., 2013a,b Rigosi et al., 2015; see Frasnelli et al., 2014 for a dedicated review) as well as on Drosophila flies (e.g., Purnell and Thompson, 1973; Gaudry et al., 2013; Buchanan et al., 2015). Furthermore, asymmetrical traits have been recently showed in several other insect species. Good examples are the courtship and mating traits of stored-product beetles (Benelli et al., 2016a, 2017b; Romano et al., 2016b), two encyrtid parasitoids (Romano et al., 2016a, 2017a) and the olive fruit fly (Benelli et al., 2015a). Lateralization during aggressive interactions has been observed in tephritid flies (Benelli et al., 2015a,c), blowflies (Romano et al., 2015), and in the Asian tiger mosquito (Benelli et al., 2015b), while asymmetrical escape and predator surveillance responses have been reported very recently for locusts exposed to Guinea-fowl mimicking predators (Romano et al., 2017c).

A theoretical model on the evolution of asymmetries suggested that lateralization at the population-level is more likely to evolve in social species, while lateralization at an individual-level is more likely to evolve in solitary species (Ghirlanda and Vallortigara, 2004; Ghirlanda et al., 2009). However, as outlined by recent evidences on the insects and spiders mentioned above, population-level asymmetric traits have been reported also in several gregarious and solitary species. It has been hypothesized that the population-level behavioral asymmetries found in these species relate to mating and other social interactions (e.g., fighting and escape responses), therefore can be explained by "pre-social" interactions occurring between members of these solitary species and their conspecifics or predators (Frasnelli et al., 2012a,b; Benelli et al., 2015a,b; Romano et al., 2017c). In this scenario, the evidence of population-level lateralized questing in I. ricinus ticks represent a peculiar finding, since the intraspecific interactions among these ticks are limited to strict time frames during their life (i.e., mating or sharing of highly infested hosts), therefore it looks conceivable to argue that the arising of population-level lateralized questing can be linked with the repeated interactions of ticks with their hosts, where lateralized questing allows ticks to enhance cognitive capacity and efficiency of the brain, thus counteracting the ecological disadvantages of lateral biases in behavior (Vallortigara and Rogers, 2005a).

4. Conclusions

Overall, to the best of our knowledge, this is the first report showing evidence of behavioral asymmetries in ticks of medical and veterinary importance, with special reference to laterality in ixodiid questing. The apparatus presented here allows a repeated and standardized presentation of the cues to the animal, thus creating repeated testing sessions in sequence, a relatively rare result in laterality literature (see Frasnelli et al., 2012a,b as well as Benelli et al., 2015a,b). Moreover, the mechatronic apparatus developed in this research can be exploited to evaluate the impact of repellent products on tick questing in highly reproducible standardized conditions, which is currently a major challenge in tick management science (Benelli et al., 2016b; Tabari et al., 2017). Further research is still needed on the proximate mechanisms leading to left-biased questing, shedding light on potential differences in muscular size, exoskeleton robustness and/or nervous innervations (see also Govind, 1989) between left and right tick anterior legs.

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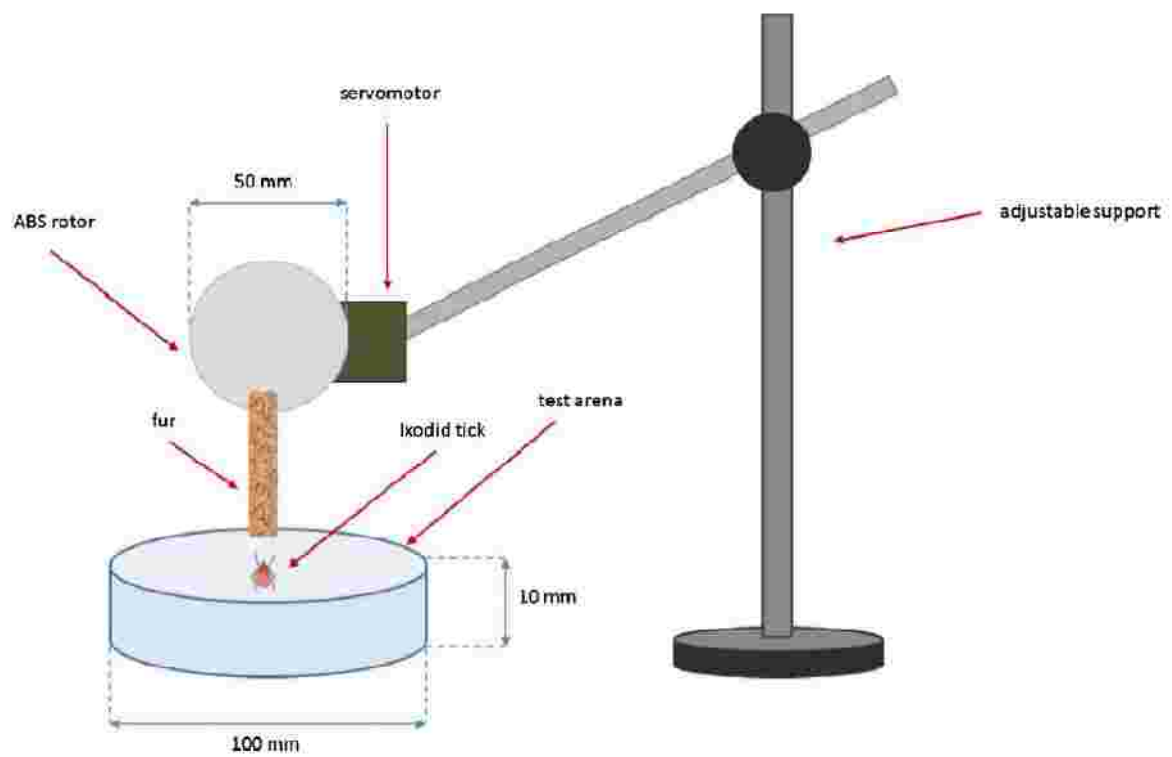
Fig. 1. The mechatronic apparatus used to deliver host-mimicking cues to *Ixodes ricinus* ticks, evoking questing behavior.
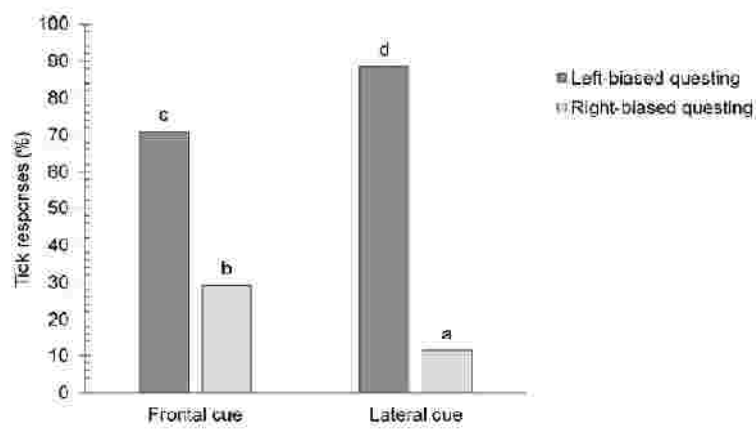
Fig. 2. Population-level questing responses of *Ixodes ricinus* adult females post exposure to host mimicking cues provided frontally or from the left or the right side of the tick, using a mechatronic apparatus. Above each column, different letters indicate significant differences (χ^2 test with Yates' correction, $P < 0.05$).

Table 1

Individual-level lateralized questing behavior in *Ixodes ricinus* ticks exposed frontally to a mechatronic apparatus delivering host-mimicking cues. To calculate the laterality index (LI), each tick was repeatedly tested thirty times.

Tick ID	Right (n)	Left (n)	LI	Individual laterality
1	12	18	− 0,2	L
2	3	27	− 0,8	L
3	7	23	− 0,5	L
4	19	11	0,3	R
5	20	10	0,3	R
6	12	18	− 0,2	L
7	15	15	0	0
8	23	7	0,5	R
9	19	11	0,3	R
10	22	8	0,5	R
11	5	25	− 0,7	L
12	7	23	− 0,5	L
13	8	22	− 0,5	L
14	6	24	− 0,6	L
15	21	9	0,4	R
16	10	20	− 0,3	L
17	10	20	− 0,3	L
18	28	2	0,9	R
19	10	20	− 0,3	L
20	9	21	− 0,4	L
21	17	13	0,1	0
22	7	23	− 0,5	L
23	11	19	− 0,3	L
24	8	22	− 0,5	L
25	3	27	− 0,8	L

LI = Laterality Index.
L = Left.
R = Right.

Table 2

Individual-level lateralized questing behavior in *Ixodes ricinus* ticks exposed to left- or right-biased host-mimicking cues delivered by a mechatronic apparatus. To calculate the laterality index (LI), each tick was repeatedly tested thirty times.

Tick ID	R-biased cue (n)	L-biased cue (n)	LI cue	Lateralized cue	R leg (n)	L leg (n)	LI leg	Lateralized leg
1	13	17	−0,1	0	8	22	−0,5	L
2	16	14	0,1	0	3	27	−0,8	L
3	13	17	−0,1	0	8	22	−0,5	L
4	12	18	−0,2	L	8	22	−0,5	L
5	14	16	−0,1	0	9	21	−0,4	L
6	18	12	0,2	R	10	20	−0,3	L
7	14	16	−0,1	0	10	20	−0,3	L
8	8	22	−0,5	L	22	8	0,5	R
9	14	16	−0,1	0	2	28	−0,9	L
10	12	18	−0,2	L	11	19	−0,3	L
11	15	15	0	0	6	24	−0,6	L
12	15	15	0	0	7	23	−0,5	L
13	16	14	0,1	0	5	25	−0,7	L
14	15	15	0	0	7	23	−0,5	L
15	22	8	0,5	R	23	7	0,5	R
16	19	11	0,3	R	8	22	−0,5	L
17	15	15	0	0	9	21	−0,4	L
18	18	12	0,2	R	8	22	−0,5	L
19	19	11	0,3	R	22	8	0,5	R
20	16	14	0,1	0	8	22	−0,5	L
21	18	12	0,2	R	10	20	−0,3	L
22	14	16	−0,1	0	3	27	−0,8	L
23	16	14	0,1	0	8	22	−0,5	L
24	15	15	0	0	7	23	−0,5	L
25	18	12	0,2	R	8	22	−0,5	L

LI = Laterality Index.

L = Left.

R = Right.