

## RESEARCH ARTICLE

# Encoding lateralization of jump kinematics and eye use in a locust via bio-robotic artifacts

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## ABSTRACT

The effect of previous exposure to lateral sensory stimuli in shaping the response to subsequent symmetric stimuli represents an important overlooked issue in neuroethology, with special reference to arthropods. In this research, we investigated the hypothesis to 'programme' jumping escape direction as well as surveillance orientation in young and adult individuals of *Locusta migratoria* as an adaptive consequence of prior exposure to directional-biased predator approaches generated by a robotic leopard gecko representing *Eublepharis macularius*. The manipulation of the jumping escape direction was successfully achieved in young locusts, although young *L. migratoria* did not exhibit innately lateralized jumping escapes. Jumping escape direction was also successfully manipulated in adult locusts, which exhibited innate lateralized jumping escape at the individual level. The innate lateralization of each instar of *L. migratoria* in using a preferential eye during surveillance was not affected by prior lateralized exposure to the robotic gecko. Our results indicate a high plasticity of the escape motor outputs that are occurring almost in real time with the perceived stimuli, making them greatly adaptable and compliant to environmental changes in order to be effective and reliable. In addition, surveillance lateralization innately occurs at population level in each instar of *L. migratoria*. Therefore, its low forgeability by environmental factors would avoid disorganization at swarm level and improve swarm coordination during group tasks. These findings are consistent with the fact that, as in vertebrates, in insects the right hemisphere is specialized in controlling fear and escape functions.

**KEY WORDS:** Animal-robot systems, Lateralization, Learning, *Locusta migratoria*, Neuroethology, Predator–prey interactions

## INTRODUCTION

Escape and surveillance responses to predators represent profitable models to study the conversion process of sensory signals into motor outputs in an organism. These behaviours have been found to be implemented by specialized neural circuits in a number of animal species (Wang and Frost, 1992; Graziano et al., 1994; Wicklein and Strausfeld, 2000; Yamamoto et al., 2003; Preuss et al., 2006; Oliva et al., 2007; Fotowat et al., 2009). However, basic knowledge on how these sensorimotor sequences are affected by visual experience and how this influences lateralization is extremely scarce.

Lateralization (i.e. the different specialization of the left and right sides of the brain, producing left–right asymmetries in the behaviour) is a fundamental principle of the brain arrangement widely described in vertebrates (Rogers et al., 2013a; Vallortigara et al., 2011; Vallortigara and Rogers, 2005; Vallortigara and Versace, 2017). Interestingly, although invertebrates have a relatively compact nervous system, lateralized traits were also identified in these species and reported by a growing number of studies (Ades and Ramires, 2002; Backwell et al., 2007; Benelli et al., 2015a,b,c; Rigosi et al., 2015; Rogers and Vallortigara, 2008, 2015; Rogers et al., 2013b, 2016; Romano et al., 2015, 2016a,b, 2018a; Benelli, 2018; Benelli and Romano, 2019). Understanding how prior context experience affects the processing of sensory stimuli by an asymmetrical nervous system (e.g. to generate complex actions such as anti-predator behaviours) can represent an important goal in computational neuroscience. Indeed, it would add significant information to formulate advanced mathematical models on visuomotor neuro-machinery involving sensory processing, brain specialization, cognition and learning. Furthermore, it can provide important insights on predator–prey interaction dynamics.

The suborder Caelifera (Orthoptera) includes remarkable biological models to assess these issues, since the brain neuro-architecture is well described in these insects (Rind, 2002; Santer et al., 2006, 2008; Kurylas et al., 2008; Fotowat et al., 2011). In particular, in Caelifera – such as locusts – each optic lobe (included in the protocerebrum) contains a neuron [the lobula giant movement detector (LGMD)] at the level of the third neuropil that responds specifically to looming stimuli (e.g. an approaching predator) (Santer et al., 2006, 2008; Fotowat et al., 2011) by producing trains of spikes transmitted to the descending contralateral movement detector (DCMD) that conveys visual information to motor centres (O'shea et al., 1974; Simmons, 1980; Fotowat et al., 2011). However, sky compass orientation (Homberg, 2004) and, as reported in flies, right–left motor coordination (Strauss and Heisenberg, 1993; Strauss, 2002), as well as visual memory (Liu et al., 2006), are regulated by the central complex, which is another area included in the protocerebrum (Fotowat et al., 2011). Locusts have been reported to be lateralized at an individual level and, interestingly, show refined motor control in the preferred forelimb (Bell and Niven, 2014, 2016). Additionally, locusts were found to be lateralized at an individual level during escape from a predator, and were lateralized at a population level during predator surveillance (Romano et al., 2017a).

An important issue concerns the basic knowledge on the network connecting sensory signals with asymmetrical motor outputs/orientations, and how they are modulated by experience. To manipulate a predator–prey interaction, we took a bio-hybrid approach, which involved developing a robotic apparatus that actuates an artificial agent mimicking a predator of locusts, the leopard gecko *Eublepharis macularius* (Blyth 1854) (Squamata: Eublepharidae) (Thorogood and Whimster, 1979; Cooper and Williams, 2014).

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In highly unstructured natural environments, many harmless objects move into these scenarios (e.g. leaves and twigs moved by the wind). Thus, it looks conceivable that prey can identify some selected cues to recognize a threat (Karplus and Algom, 1981; Honma et al., 2006) to escape only if necessary and reduce the cost of the escape (Ydenberg and Dill, 1986). A number of researches relied on biomimetic dummies resembling real predators to study lateralization of the escape responses (Facchin et al., 1999; Lippolis et al., 2002, 2005), staging a more likely predator–prey interaction. Many other studies have successfully elicited escapes with looming disks in several animal species, such as frogs and insects (e.g. Ingle, 1973; Schlotterer, 1977; Santer et al., 2005; Peron and Gabbiani, 2009; Rodriguez-Romaguera and Stuber, 2018).

However, the emerging scientific field of ethorobotics offers new paradigms of experimental manipulations of intraspecific and interspecific interactions (Todd, 1993; Webb, 1995, 2000; Halloy et al., 2007; Partan et al., 2009; Krause et al., 2011; Romano et al., 2017a). Therefore, ethorobotics enables the achievement of highly standardized and controllable testing in animal behavioural research, by avoiding mutual influences and/or feedbacks between multiple animals (Herbert-Read et al., 2012; Jolles et al., 2017; Harcourt et al., 2009).

Furthermore, biologically inspired robots exhibit 3-dimensional life-like cues that can be perceived more realistically by animals (Polverino et al., 2012; Halloy et al., 2013; Romano et al., 2017b, 2018b; Bonnet et al., 2018; Kim et al., 2018; Bierbach et al., 2018), compared with other synthetic approaches (Tinbergen, 1951; Lippolis et al., 2002; Woo and Rieucou, 2011). Conversely, the study of living organisms with this bio-hybrid strategy can leverage robot design and improve the development of advanced bioinspired artifacts that efficiently perceive the environment and behave within it (Ijspeert et al., 2005; Wood, 2008; Li et al., 2012; Stefanini et al., 2012; Bonsignori et al., 2013; El Daou et al., 2014; Daler et al., 2015).

Herein, gregarious individuals of *Locusta migratoria* (Linnaeus 1758) (Orthoptera: Acrididae) were trained by using a robotic leopard gecko in order to observe directional-biased predator approaches. Since antipredator behaviour importantly contributes to survival and fitness boosting in animals, we investigated the hypothesis that we could ‘programme’ the locust jumping escape direction as well as surveillance orientation as an adaptive consequence of prior exposure to the robotic predator in lateralized training sessions. Furthermore, the jumping escape lateralization in locusts is age-related, while surveillance asymmetry is equally exhibited among different instars (Romano et al., 2017a). Based on this, herein we evaluated whether the developmental stage has a sensible role in the modulation of antipredator responses in this species.

## MATERIALS AND METHODS

### Ethic statements

This research adheres to the guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS, 2014) as well as the Italian and EU laws (D.M. 116192 and European Commission, 2007, respectively). All experiments are behavioural observations, and no specific permits are required in the country where the experiments were conducted.

### Insect rearing and general observations

Locusts were fed *ad libitum* with wheat, vegetables and water (Bell and Niven, 2016), and maintained at  $25\pm 1^\circ\text{C}$ ,  $55\pm 5\%$  relative humidity (R.H.) with a 16 h:8 h light:dark photoperiod. Second-instar, fourth-instar and adult *L. migratoria* individuals of both

sexes were tested. Experiments were conducted in the laboratory under the same experimental conditions described above. Light intensity around the test arena was about 1000 lux. The behaviour of locusts was directly recorded by an observer during the experiments (Benelli et al., 2015d; Romano et al., 2017a). A white wall of filter paper (Whatman) surrounded the arena and the observer was dressed in a white coat to minimize his impact on *L. migratoria* behaviour (Romano et al., 2016b, 2017a).

### Robotic leopard gecko and experimental apparatus

A gecko replica of *E. macularius* was designed in SolidWorks (Dassault Systemes, Vélizy-Villacoublay, France) and fabricated by rapid prototyping in acrylonitrile butadiene styrene (ABS). The biomimetic morphology of the *E. macularius* replica includes the head with the mouth, two eyes and nostrils, main body and the two forelegs with feet. The leopard gecko replica has a total length of 107 mm and a total width of 44 mm, reasonably corresponding to the size of *E. macularius* (Kratovil and Frynta, 2002), as it reproduces only a portion of the body of the authentic lizard.

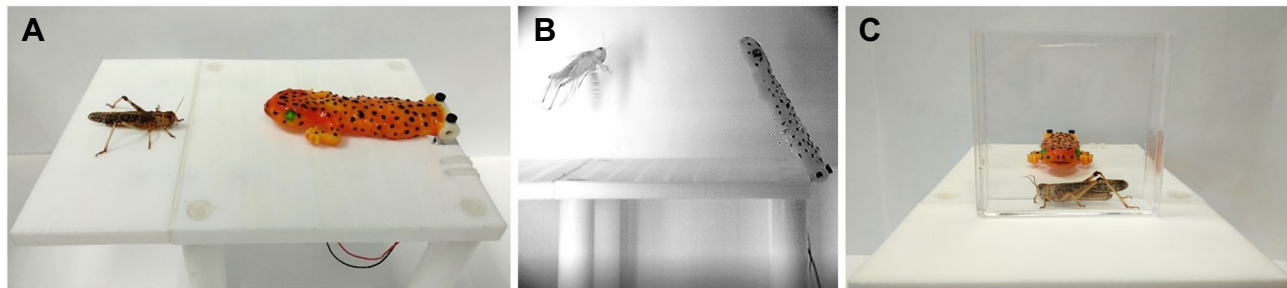
To produce a similar colour pattern and the rough skin texture of a real *E. macularius*, the artifact was endowed with a skin-like coating, obtained by mixing a transparent liquid silicone rubber (Dragon Skin, Smooth-On, Pennsylvania, USA) with nontoxic pigments and used to cover the leopard gecko replica by turning (rotational) moulding (Romano et al., 2017a). The leopard gecko replica was connected with a DC gearmotor (model: 212-103), forming a robotic arm.

In order to integrate the robotic platform, the leopard gecko replica was inserted in a dedicated slot in the middle of one of the shorter sides of a support. The support includes a flat top surface (150×220 mm), and four cylindrical legs (120 mm, diameter 20 mm) in polyoxymethylene (POM), fabricated using a computer numerical control (CNC) machine.

When the DC gearmotor is activated, the leopard gecko replica can be vertically rotated from the bottom of the test bench to the horizontal plane on the top surface. Even if one can argue that this movement did not carbon-copy an attack by a living gecko, the predatory event displayed by the robotic leopard gecko was perfectly symmetric in appearance and movement to avoid any lateral bias in locusts during the experiments. Fig. 1A and B show the robotic leopard gecko lying on the flat top surface with a locust frontally placed, as well as the jumping escape of a locust during the robotic leopard gecko approach, respectively. The activation of the leopard gecko replica’s attack was performed via a microcontroller (Arduino, Mega 2560).

### Training phase

Locusts were individually released in a transparent cage (80×15×80 mm for adults; 40×15×80 mm for neanids and nymphs), wide enough to prevent the constraining of their natural posture but significantly reducing insect locomotion. This enabled us to impose a given orientation during the simulated attacks by the robotic leopard gecko. The transparent cage containing the locust was placed on the top surface of the test bench, perpendicularly to the longitudinal body axis of the robotic leopard gecko at a distance >70 mm from its mouth when it lies on the horizontal plane (see Fig. 1C). The posture of *L. migratoria* was constantly monitored during the training phase to ensure correct orientation. The training phase lasted 60 min, in which the robotic leopard gecko appeared from the bottom of the test bench (not visible to the locust) to the horizontal plane of the top surface, and then returned, at intervals of 30 s. According to the side of the locust that was



**Fig. 1. The animal-robot interaction.** (A) The robotic leopard gecko and a locust (*Locusta migratoria*) on the top surface of the experimental apparatus. (B) Snapshot of a bio-hybrid predator-prey interaction, showing the approaching robotic leopard gecko and an escaping locust. (C) A locust in the transparent cage with the right side exposed to the predator-mimicking robot during the training phase.

exposed to the simulated predator, we obtained left/right-trained locusts.

It is well acknowledged that just the risk of predation can produce physiological stresses in a wide number of species. These stresses have been reported to have long-lasting influences on prey escape responses (Cannon, 1915; Selye, 1936; Hawlena et al., 2011). Thus, although the robotic leopard gecko did not physically injure the locusts (this would not be acceptable from an ethical point of view), its presence as a potential predator and its simulated attack would affect the locust escape response.

Before training a new locust, the whole setup was rotated manually 90 deg horizontally in a clockwise direction, to randomize its orientation and to avoid positional effects (Canale et al., 2015). Therefore, at the end of a training we removed the locust and rotated the setup. Soon after the rotation of the setup, a new locust was introduced.

The control treatments were similarly conducted, with the exceptions that the robotic leopard gecko was not visible to the locusts (i.e. hidden below the test bench), and no simulated attacks were displayed. The interval between the training phase and the experiments described below was 120 min, during which locusts were isolated from other subjects to avoid subsequent experiences affecting the effect of the training.

### Experiment 1: modulating the jumping escape lateralization

Here, the possibility to condition the direction of the jumping escape response by providing a symmetric stimulus to previously trained locusts was evaluated. *Locusta migratoria* were trained by presentation of the robotic stimulus to the left or right compound

eye, and were placed individually [as were control subjects (naïve)] on the top surface of the test bench, with their longitudinal body axis accurately centred ( $\pm 0.5$  deg) with the longitudinal body axis of the robotic leopard gecko at a distance of  $\approx 70$  mm from its mouth when it lies on the horizontal plane. The robotic apparatus was placed in the centre of a rectangular white arena ( $800 \times 600 \times 600$  mm), equidistant from the left and right side, to minimize external cues affecting the locust's behaviour. At the beginning of the test, the robotic leopard gecko was hidden below the test bench and thus not visible to the tested locust.

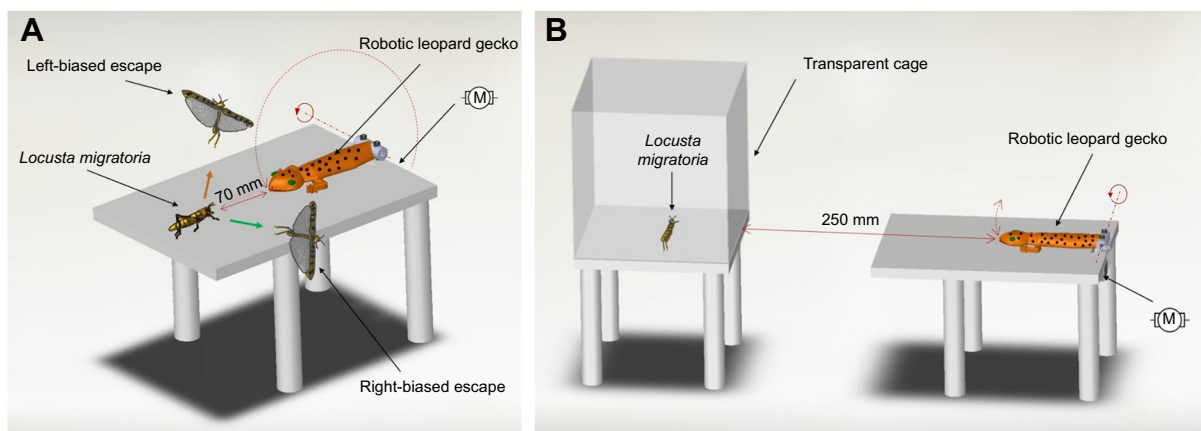
The jumping escape direction of the locust was recorded following the robotic leopard gecko rotation from the bottom of the test bench to the horizontal plane on the top surface ( $\omega = 4.97$  rad  $s^{-1}$ ), emulating a predator that comes out of a hiding place (see Fig. 2A). Locusts that were not accurately centred with the robotic leopard gecko when approached by it were not considered for laterality observations.

For each replicate, the whole setup was rotated manually as described in the 'Training phase' paragraph, to avoid positional effects.

For each developmental stage of *L. migratoria* considered in our study (e.g. second instar, fourth instar and adult), 25 naïve subjects, 25 left-trained subjects and 25 right-trained subjects, escaping after a perfectly symmetric predator-prey interaction, were analyzed. The direction of 30 jumps, delayed 10 min from each other, was recorded for each insect (Romano et al., 2017a).

### Experiment 2: modulating the surveillance lateralization

Here, the assumption to manipulate the eye use preference during surveillance in locusts by a prior bio-robotic interaction was



**Fig. 2. Experimental setup.** Schematic illustrations of (A) experiment 1 and (B) experiment 2.



evaluated. A transparent cubic cage (150×150×150 mm) containing a locust was placed in the middle of a rectangular white arena (1300×900×600 mm) at the same distance from the right and left side of the arena. The cubic cage was positioned at 250 mm from the robotic leopard gecko mouth when it lies on the horizontal plane. The floor of the cubic cage and the top surface of the test bench were positioned on the same horizontal plane.

Before starting the test, the robotic leopard gecko was not visible to the tested locust since it was hidden below the test bench. After 5 min from the introduction of the locust inside the transparent cage, the test started, and the robotic leopard gecko rotated from the bottom of the test bench to the horizontal plane on the top surface. Then, for the whole duration of this experiment, a still phase lasting 15 s, in which the robotic leopard gecko was motionless on the top surface, was alternated with a pitch phase, lasting 5 s. During the pitch phase, the robotic leopard gecko rotated 30 deg from bottom to top and then returned (5 Hz) (see Fig. 2B). The larger distance of the robotic agent from the locust, compared with the experiment 1, and the short angle and duration of the pitch phases ensured to better select cryptic and surveillance behaviours (Eterovick et al., 1997; Ruxton et al., 2004; Ruxton, 2006) and to avoid an excessive threat triggering an escape response.

The test lasted 30 min, and the exposure duration of orientation of each side of the locust body to the robotic leopard gecko was recorded for each animal by following the method by Romano et al. (2017a). In particular, to ensure the monocular vision of the robot by the locust, only locusts with their steered body axis forming an angle >45 deg with the initial orientation of their body axis, aligned with the stimulus, were considered for laterality observations (Horridge, 1977; Kral and Poteser, 1997). For each replicate, the whole setup was rotated manually as described in the ‘Training phase’ paragraph, to avoid positional effects. A total of 25 naïve subjects, 25 left-trained subjects and 25 right-trained subjects were tested for each *L. migratoria* developmental stage observed in this research (e.g. second instar, fourth instar and adult).

### Statistical analysis

To analyze the differences in the direction of jumping escape responses, as well as in the use of the right and left eye during predator surveillance, a laterality index (LI) was calculated for each insect, following the method by Frasnelli et al., (2012):  $LI=(R-L/R+L)$ .

For the predator escape behaviour, *R* and *L* indicate, respectively, the normalized mean number of times in which each locust jumped to the right or to the left. A score of 1.0 indicated exclusive preference to jump to the right, while a score of -1.0 indicated exclusive preference to jump to the left. A score of 0 indicated equal numbers of right and left jumps during jumping escape acts.

For the predator surveillance behaviour, *R* and *L* indicate, respectively, the normalized mean value of the duration in which each locust used the right eye or the left eye to oversee the robotic predator. A score of 1.0 indicated exclusive use of the right eye, while a score of -1.0 indicated exclusive use of the left eye. A score of 0 indicated equal duration in using the right and left eye during surveillance.

Furthermore, the absolute value of the laterality index (ABLI) was considered, to discriminate individuals with a bilateral dominance from individuals with a lateral dominance, regardless of the left or the right direction of the bias, and to evaluate the strength of lateralization (Bisazza et al., 2000; Seghier, 2008; Romano et al., 2017a).

Laterality differences among naïve, right-trained and left-trained locusts over different instars (second young instars, fourth young

instars and adults) displaying right- or left-biased jumping escapes, as well as right- or left-biased eye use during surveillance, were analyzed by JMP 9 (SAS) using a general linear model with two factors, i.e. the tested naïve/trained instar and laterality.  $P<0.05$  was used to assess the significance of differences between means. Moreover, for each treatment, the difference in the number of locusts jumping to the right or left as well as using left or right eyes during the interaction with the biomimetic gecko predator was analyzed using a  $\chi^2$  test with Yates' correction ( $P<0.05$ ).

## RESULTS

### Experiment 1: modulating the jumping escape lateralization

The direction of motor outputs towards left or right during the jumping escape from the robotic leopard gecko was successfully manipulated in all locust instars following exposure in the training phase, thus allowing to control the lateralization of the jumping escape at a population level. The population mean value of the jumping escape's LI was significantly modulated by different contexts of the training phase ( $F_{8,224}=41.077$ ;  $P<0.0001$ ). The LI of each left-trained locust instar shows the preference to jump to the right. The LI of each naïve instar shows an equal preference of these locusts to jump to the right and to the left. The LI of each right-trained locust instar shows the preference to jump to the left (Fig. 3A, Table S1).

The ABLI was significantly modulated by the training phase ( $F_{8,224}=31.684$ ;  $P<0.0001$ ). In adults, the ABLI was marginally higher in naïve subjects compared with right-trained locusts, and significantly higher compared with left-trained locusts. In fourth instar locusts, the ABLI of both right-trained and left-trained subjects was significantly higher compared with the ABLI of naïve locusts. In second instar locusts, the ABLI of right-trained subjects as well as left-trained insects was significantly higher compared with the ABLI of naïve locusts (Fig. 3B, Table S1).

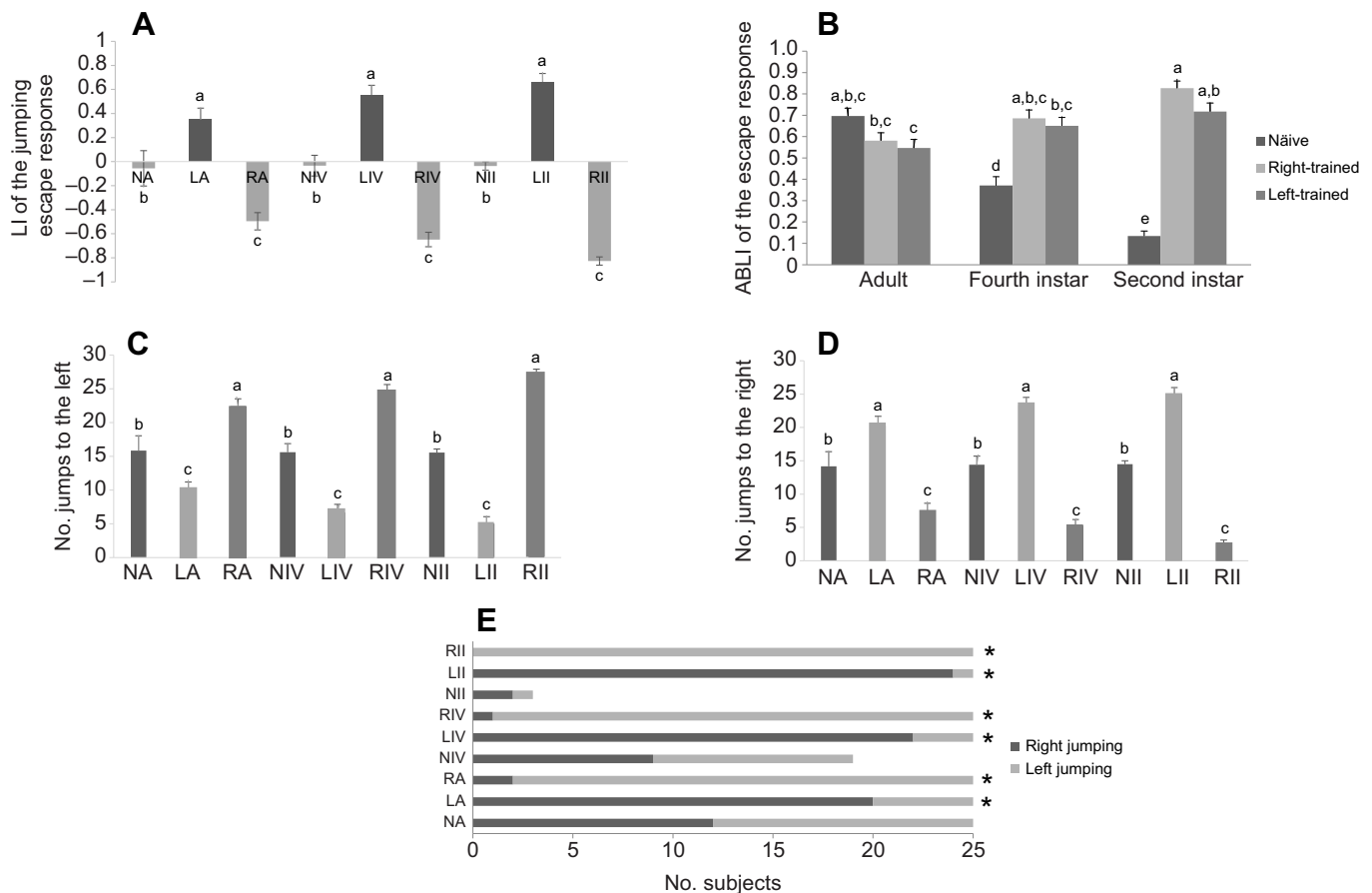
The number of jumps to the left was importantly affected by the training phase ( $F_{8,224}=41.080$ ;  $P<0.0001$ ). For each locust instar considered, the number of left jumps was higher in right-trained insects compared with naïve individuals. In addition, the number of left jumps was lower in left-trained insects compared with naïve individuals (Fig. 3C, Table S1).

The number of jumps to the right was significantly affected by the training phase ( $F_{8,224}=41.071$ ;  $P<0.0001$ ). Regardless of the tested locust instars, the number of right jumps was higher in left-trained individuals compared with naïve individuals, and the number of right jumps was lower in right-trained locusts compared with naïve individuals (Fig. 3D, Table S1).

The lateralization of the jumping escape at population level was successfully determined in each locust instar involved in a training context (Fig. 3E, Table S1). The number of naïve adult locusts that preferentially jumped to the left did not significantly differ from the number of naïve adult locusts that preferentially jumped to the right (left versus right: 13 versus 12;  $\chi^2_1=0.001$ ;  $P=0.99$ ). The same results were observed testing naïve fourth instar locusts (left versus right: 10 versus 9;  $\chi^2_1=0.001$ ;  $P=0.99$ ), as well as naïve second instar locusts (left versus right: 2 versus 1;  $\chi^2_1=0.001$ ;  $P=0.99$ ).

Right-trained locusts preferentially jumped to the left when approached by the robotic-borne combination of cues, in each instar considered, including adult locusts (left versus right: 23 versus 2;  $\chi^2_1=16$ ;  $P<0.0001$ ), fourth instar locusts (left versus right: 24 versus 1;  $\chi^2_1=19.36$ ;  $P<0.001$ ) and second instar locusts (left versus right: 25 versus 0;  $\chi^2_1=23.04$ ;  $P<0.001$ ) (Table S1).

Left-trained locusts preferentially jumped to the right when approached by the robotic leopard gecko. This was noted for adult locusts (left versus right: 5 versus 20;  $\chi^2_1=7.84$ ;  $P=0.0051$ ), fourth



**Fig. 3. Experiment 1: laterality index (LI), absolute value of the laterality index (ABLI) and direction bias in jumping escape.** (A) LI, (B) ABLI, (C) left- and (D) right-biased jumps characterizing the jumping escape response of different trained *L. migratoria* during the symmetric exposure to the robotic leopard gecko. Lowercase letters near each bar indicate significant differences ( $P < 0.05$ ). T-bars represent s.e.m. (E) Left- and right-biased *L. migratoria* showing a lateralized jumping escape during the symmetric exposure to a robotic leopard gecko. Asterisks indicate significant differences between left- and right-biased locusts ( $P < 0.05$ ). NA, naïve adult; LA, left-trained adult; RA, right-trained adult; NIV, naïve fourth instar; LIV, left-trained fourth instar; RIV, right-trained fourth instar; NII, naïve second instar; LII, left-trained second instar; RII, right-trained second instar.

instar locusts (left versus right: 3 versus 22;  $\chi^2_1 = 12.96$ ;  $P = 0.0003$ ) and second instar locusts (left versus right: 1 versus 24;  $\chi^2_1 = 19.36$ ;  $P < 0.0001$ ) (Table S1).

### Experiment 2: modulating the surveillance lateralization

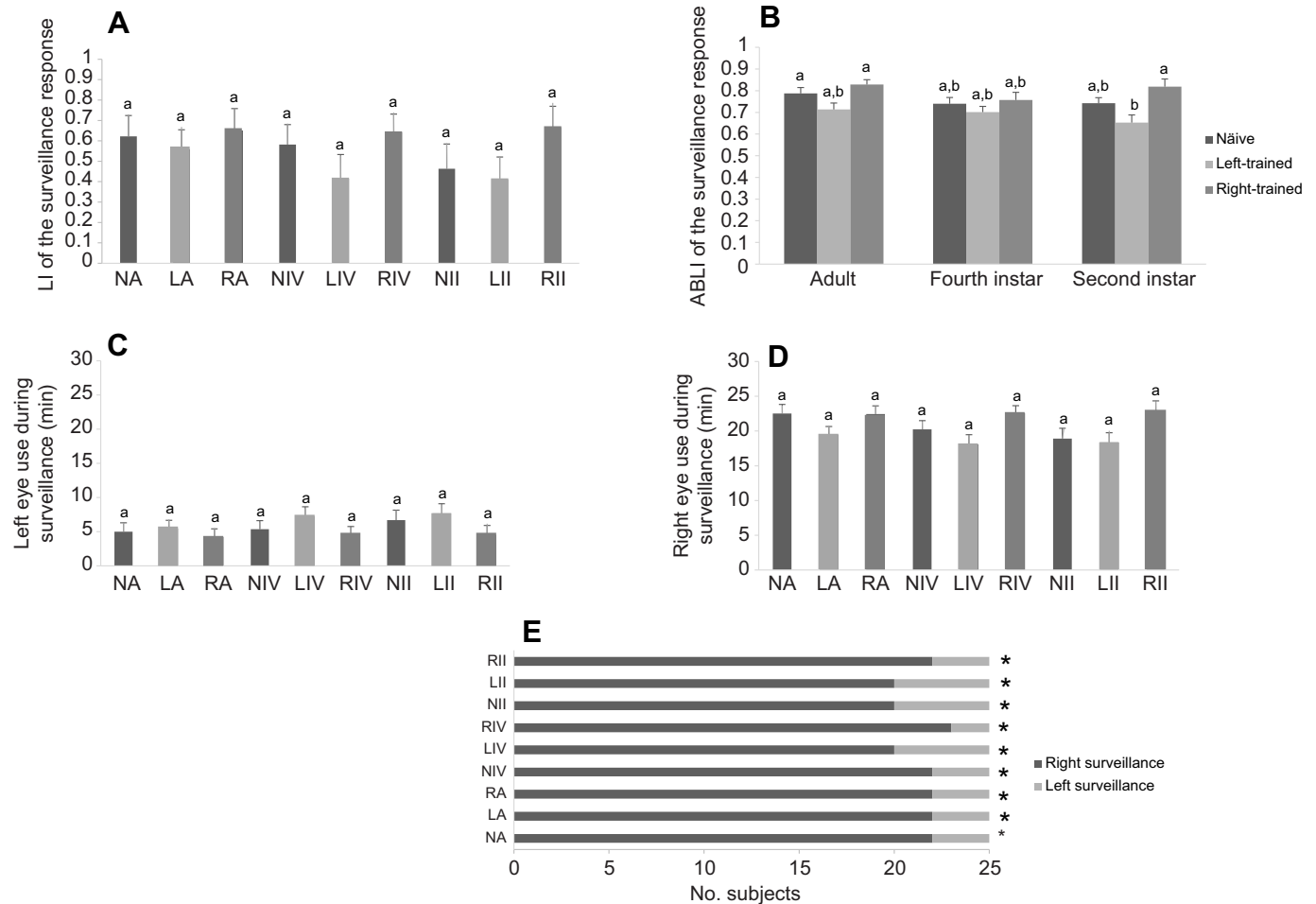
This experiment showed innate lateralization of *L. migratoria* using a preferential eye during surveillance. The poor plasticity of this feature to adapt to environmental perturbations (e.g. a predator-mimicking robotic stimulus) was also reported. The population mean value of the LI of eye use was not significantly influenced by different contexts of the training phase ( $F_{8,224} = 0.936$ ;  $P = 0.486$ ). Each training phase produced right-biased locusts, regardless of the instar of the insects (Fig. 4A, Table S2).

The ABLI was marginally modulated by the training phase ( $F_{8,224} = 3.585$ ;  $P = 0.0006$ ). Compared with naïve subjects of each developmental stage, only left-trained adults, as well as left-trained and right-trained second instar locusts, had a marginally different ABLI (Fig. 4B, Table S2).

The duration of time intervals in which each locust used the left eye to oversee the robotic predator was not importantly affected by the training phase ( $F_{8,224} = 0.919$ ;  $P = 0.501$ ). For each locust instar, the duration of the left eye use to oversee the robotic leopard gecko was similar for naïve, left-trained and right-trained subjects (Fig. 4C, Table S2).

The duration of time intervals in which each locust used the right eye to oversee the robotic predator was marginally affected by the training phase ( $F_{8,224} = 1.899$ ;  $P = 0.061$ ). Naïve, left-trained and right-trained locusts performed the surveillance with the right eye with a similar duration in each locust instar considered (Fig. 4D, Table S2).

Surveillance lateralization at population level was not affected by training (Fig. 4E, Table S2). The number of naïve adults (left versus right: 3 versus 22;  $\chi^2_1 = 12.96$ ;  $P = 0.00031$ ), left-trained adults (left versus right: 3 versus 22;  $\chi^2_1 = 12.96$ ;  $P = 0.00031$ ) and right-trained adults (left versus right: 3 versus 22;  $\chi^2_1 = 12.96$ ;  $P = 0.00031$ ) that preferentially used the right eye to oversee the robotic leopard gecko was significantly higher compared with naïve, left-trained and right-trained adults that preferentially used the left eye during surveillance (Table S2). Also, naïve fourth instar locusts (left versus right: 3 versus 22;  $\chi^2_1 = 12.96$ ;  $P = 0.0003$ ), left-trained fourth instar locusts (left versus right: 5 versus 20;  $\chi^2_1 = 7.84$ ;  $P = 0.0051$ ) and right-trained fourth instar locusts (left versus right: 2 versus 23;  $\chi^2_1 = 16$ ;  $P < 0.0001$ ), as well as naïve second instar locusts (left versus right: 5 versus 20;  $\chi^2_1 = 7.84$ ;  $P = 0.0051$ ), left-trained second instar locusts (left versus right: 5 versus 20;  $\chi^2_1 = 7.84$ ;  $P = 0.0051$ ) and right-trained second instar locusts (left versus right: 3 versus 22;  $\chi^2_1 = 12.96$ ;  $P = 0.00031$ ), preferentially used the right eye to oversee the robotic leopard gecko (Table S2).



**Fig. 4. Experiment 2: LI, ABLI and direction bias in surveillance.** (A) LI, (B) ABLI, (C) left- and (D) right-biased eye use characterizing surveillance of different trained *L. migratoria* during the symmetric exposure to the robotic leopard gecko. Lowercase letters near each bar indicate significant differences ( $P < 0.05$ ). T-bars represent s.e.m. (E) Left- and right-biased *L. migratoria* during the surveillance of a robotic leopard gecko. Asterisks indicate significant differences between left- and right-biased locusts ( $P < 0.05$ ). NA, naïve adult; LA, left-trained adult; RA, right-trained adult; NIV, naïve fourth instar; LIV, left-trained fourth instar; RIV, right-trained fourth instar; NII, naïve second instar; LII, left-trained second instar; RII, right-trained second instar.

## DISCUSSION

How earlier lateral sensory stimuli, for example generated by an approaching artifact mimicking a predator, are processed by the nervous system, and how they produce a lateralized behaviour in the subsequent presence of symmetric stimuli, represents a novel and important area of investigation in relation to the evolutionary neuro-behavioural ecology of a species. Here, we used *L. migratoria* as a model organism, since the locust nervous system has been deeply investigated (Rind, 2002; Santer et al., 2006; 2008; Kurylas et al., 2008; Fotowat et al., 2011), reporting these insects as innately lateralized in several behaviours (Bell and Niven, 2014, 2016; Romano et al., 2017a). To maximize the standardization and accuracy of the experimental conditions in such delicate testing contexts, a remarkable role is played by robotic agents, which enable researchers to establish bio-hybrid interactions with animals (Todd, 1993; Webb, 1995, 2000; Halloy et al., 2007; Partan et al., 2009; Krause et al., 2011; Polverino et al., 2012; Romano et al., 2017a,b; Bonnet et al., 2018; Kim et al., 2018; Bierbach et al., 2018). However, further efforts are needed to provide an in-depth analysis comparing the effect of different strategies used to provide stimuli evoking antipredator behaviours (Dill, 1974).

The results reported in this study offer new and fascinating insights on how a lateralized brain reacts and adapts to dynamic

events that are crucial for survival, such as predator–prey interactions (Bonsall and Hassell, 2007; Millon and Bretagnolle, 2008; Dessborn et al., 2009). The most interesting fact is that the same visual cues (e.g. robotic-borne stimuli), laterally perceived during the training phase, were able to influence the jumping direction of subsequent escape responses to symmetric stimuli, but they did not affect the preferential eye use for surveillance (Figs 3 and 4). This indicates a high plasticity of those escape motor outputs that are occurring almost in real time with the perceived stimuli, making them greatly adaptable and compliant to environmental changes in order to be effective and reliable. In particular, we observed that locusts can adapt the direction of the jumping escape to external asymmetric events repeatedly occurring, and to maintain this preference when no stimuli or symmetric stimuli are presented.

We found that the main factor conditioning training is represented by vision, directing sensory information to motor centres and likely producing motor learning at the level of the prothoracic ganglion of locusts (Horridge, 1962; Rowell, 1961, 1964). In fact, motor action was not involved, because of external constraints that prevented jumping escapes. Although insects could make limited movements inside the cage during the training phase, they could not display a real jumping escape.

It has been reported that several species are right-biased during escape responses, while others are left-biased (Cantalupo et al., 1995; Lippolis et al., 2009; Bonati et al., 2010). In populations with a high risk of predation, prey prefer to observe the predator with a given eye over the other (Brown et al., 2004). Several vertebrate species exhibit a higher reactivity when they are approached by predators from their left visual field (controlled by their right hemisphere) (Lippolis et al., 2002, 2005; Austin and Rogers, 2007), if compared to the right visual field. These studies confirm that, in vertebrates, the right hemisphere is specialized for the control of fear and escape responses (Lippolis et al., 2005), potentially representing a phylogenetic ancient trait (Lippolis et al., 2002). Interestingly, our results are consistent with this right-hemisphere specialization, although the biological model here was an invertebrate. Indeed, in insects, each compound eye is connected with the ipsilateral optic lobe included in the protocerebrum (Strausfeld, 2005). Therefore, since locusts preferentially used the right compound eye for surveillance, we can assume that also in insects the right hemisphere of the cerebrum controls fear and escape functions.

In addition, surveillance lateralization has been found to have a strict programme to adhere to. This is probably part of a higher level of brain organization that includes other functions to be carried out in parallel (Vallortigara and Rogers, 2005; Frasnelli, 2013; Romano et al., 2017a). Furthermore, surveillance lateralization is a population-level feature innately occurring in *L. migratoria* aggregations, as reported by earlier research (Romano et al., 2017a): its low forgeability by environmental factors would avoid disorganization at swarm level and would improve swarm coordination during group tasks, as also confirmed by findings on other gregarious species (Chivers et al., 2016).

The highly lateralised response of young instars after the training with a leopard-gecko-mimicking robot is particularly interesting, although naïve young individuals do not exhibit innately lateralized jumping escapes (Romano et al., 2017a). This is in contrast with recent evidence in rats (Kurzina et al., 2018), where lateralized motor behaviours in young subjects were little affected by previous learning compared to adults. A possible explanation is consistent with the hypothesis that young individuals belonging to several mammalian species are largely depending on parental care. These mammal species first develop cortical functions such as perception, language and cognition (Merzenich, 2001; Cornelissen et al., 2004; Shtyrov et al., 2010); consequently, motor learning is developed slower. Although young locusts do not present innately lateralized traits in the jumping escape, they have shown an impressive reactive motor learning system producing lateralized adaptive behaviours. This is probably due to their greater vulnerability to predation compared with adults (Gillett and Gonta, 1978), an aspect that is shared with many other prey species (Geist, 1971; Fitzgibbon, 1990).

The successful induction of lateralized jumping escape in young locusts, besides adults, can be related to an antipredator tactic based on early motor learning to environmental factors. Further efforts are needed to understand how motor learning is affected by lateralized stimuli in young insects belonging to species exhibiting parental care. Our findings add novel insights to different hypothesis on lateralization in vertebrates and invertebrates that would be determined by a common ancestor or by convergent evolution (Ghirlanda and Vallortigara, 2004; Frasnelli, 2013).

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: D.R.; Methodology: D.R., C.S.; Validation: D.R., G.B., C.S.; Formal analysis: D.R.; Investigation: D.R., G.B.; Resources: C.S.; Data curation: D.R., G.B., C.S.; Writing - original draft: D.R.; Writing - review & editing: D.R., G.B., C.S.; Supervision: C.S.; Project administration: C.S.

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#### References

- Ades, C. and Ramires, E. N. (2002). Asymmetry of leg use during prey handling in the spider *Scytodes globula* (Scytodidae). *J. Insect Behav.* **15**, 563-570.
- ASAB/ABS (2014). Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.* **99**, 1-9.
- Austin, N. P. and Rogers, L. J. (2007). Asymmetry of flight and escape turning responses in horses. *Lateralit* **12**, 464-474.
- Backwell, P. R. Y., Matsumasa, M., Double, M., Roberts, A., Murai, M., Keogh, J. S. and Jennions, M. D. (2007). What are the consequences of being left-clawed in a predominantly right-clawed fiddler crab? *Proc. Roy. Soc. Lond. B Biol.* **274**, 2723-2729.
- Bell, A. T. A. and Niven, J. E. (2014). Individual-level, context-dependent handedness in the desert locust. *Curr. Biol.* **24**, 382-383.
- Bell, A. T. A. and Niven, J. E. (2016). Strength of forelimb lateralization predicts motor errors in an insect. *Biol. Lett.* **12**, 20160547.
- Benelli, G. (2018). Mating behavior of the West Nile virus vector *Culex pipiens*—role of behavioral asymmetries. *Acta Trop.* **179**, 88-95.
- Benelli, G. and Romano, D. (2019). Looking for the right mate—What we really know on the courtship and mating of *Lucilia sericata* (Meigen)? *Acta Trop.* **189**, 145-153.
- Benelli, G., Donati, E., Romano, D., Stefanini, C., Messing, R. H. and Canale, A. (2015a). Lateralisation of aggressive displays in a tephritid fly. *Naturwissenschaften* **102**, 1251.
- Benelli, G., Romano, D., Messing, R. H. and Canale, A. (2015b). First report of behavioural lateralisation in mosquitoes: right-biased kicking behaviour against males in females of the Asian tiger mosquito, *Aedes albopictus*. *Parasitol. Res.* **114**, 1613-1617.
- Benelli, G., Romano, D., Messing, R. H. and Canale, A. (2015c). Population-level lateralized aggressive and courtship displays make better fighters not lovers: evidence from a fly. *Behav. Process.* **115**, 163-168.
- Benelli, G., Romano, D., Desneux, N., Messing, R. H. and Canale, A. (2015d). Sex differences in fighting-induced hyperaggression in a fly. *Anim. Behav.* **104**, 165-174.
- Bierbach, D., Lukas, J. A., Bergmann, A., Elsner, K., Höhne, L., Weber, C., Weimar, N., Arias-Rodriguez, L., Mönck, H. J., Nguyen, H. et al. (2018). Insights into the social behavior of surface and cave-dwelling fish (*Poecilia mexicana*) in light and darkness through the use of a biomimetic robot. *Front. Robot. AI* **5**, 3.
- Bisazza, A., Facchin, L. and Vallortigara, G. (2000). Heritability of lateralization in fish: concordance of right-left asymmetry between parents and offspring. *Neuropsychologia* **38**, 907-912.
- Bonati, B., Csermely, D., López, P. and Martín, J. (2010). Lateralization in the escape behaviour of the common wall lizard (*Podarcis muralis*). *Behav. Brain Res.* **207**, 1-6.
- Bonnet, F., Gribovskiy, A., Halloy, J. and Mondada, F. (2018). Closed-loop interactions between a shoal of zebrafish and a group of robotic fish in a circular corridor. *Swarm Intell.* **12**, 227-244.
- Bonsall, M. B. and Hassell, M. P. (2007). Predator-prey interactions. In *Theoretical Ecology Principles and Applications*, 3rd edn, pp. 46-61. Oxford: Oxford University Press.
- Bonsignori, G., Stefanini, C., Scarfogliero, U., Mintchev, S., Benelli, G. and Dario, P. (2013). The green leafhopper, *Cicadella viridis* (Hemiptera, Auchenorrhyncha, Cicadellidae), jumps with near-constant acceleration. *J. Exp. Biol.* **216**, 1270-1279.
- Brown, C., Gardner, C. and Braithwaite, V. A. (2004). Population variation in lateralized eye use in the poeciliid *Brachyraphis episcopi*. *Proc. R. Soc. Lond. B Biol. Sci.* **271**, S455-S457.
- Canale, A., Benelli, G., Germinara, G. S., Fusini, G., Romano, D., Rapolini, F., Desneux, N., Rotundo, G., Raspi, A. and Carpita, A. (2015). Behavioural and



- electrophysiological responses to overlooked female pheromone components in the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae). *Chemoecology* **25**, 147-157.
- Cannon, W. B.** (1915). *Bodily Changes in Pain, Hunger, Fear and Rage: an Account of Recent Researches into the Function of Emotional Excitement*. New York: D Appleton & Company.
- Cantalupo, C., Bisazza, A. and Vallortigara, G.** (1995). Lateralization of predator-avoidance response in a teleost fish (*Girardinus falcatus*). *Neuropsychologia* **33**, 1637-1646.
- Chivers, D. P., McCormick, M. I., Allan, B. J. M., Mitchell, M. D., Gonçalves, E. J., Bryshun, R. and Ferrari, M. C. O.** (2016). At odds with the group: changes in lateralization and escape performance reveal conformity and conflict in fish schools. *Proc. R. Soc. B* **283**, 20161127.
- Cooper, J. E. and Williams, D. L.** (2014). The feeding of live food to exotic pets: issues of welfare and ethics. *J. Exot. Pet Med.* **23**, 244-249.
- Cornelissen, K., Laine, M., Renvall, K., Saarinen, T., Martin, N. and Salmelin, R.** (2004). Learning new names for new objects: cortical effects as measured by magnetoencephalography. *Brain Lang.* **89**, 617-622.
- Daler, L., Mintchev, S., Stefanini, C. and Floreano, D.** (2015). A bioinspired multi-modal flying and walking robot. *Bioinspir. Biomim.* **10**, 016005.
- Dessborn, L., Elmberg, J., Nummi, P., Pöysä, H. and Sjöberg, K.** (2009). Hatching in dabbling ducks and emergence in chironomids: a case of predator-prey synchrony? *Hydrobiologia* **636**, 319-329.
- Dill, L. M.** (1974). The escape response of the zebra danio (*Brachydanio rerio*) I. The stimulus for escape. *Anim. Behav.* **22**, 711-722.
- Ei Daou, H., Salumäe, T., Chambers, L. D., Megill, W. M. and Kruusmaa, M.** (2014). Modelling of a biologically inspired robotic fish driven by compliant parts. *Bioinspir. Biomim.* **9**, 016010.
- Eterovick, P. C., Figueira, J. E. C. and Vasconcellos-Neto, J.** (1997). Cryptic coloration and choice of escape microhabitats by grasshoppers (Orthoptera: Acrididae). *Biol. J. Linn. Soc.* **61**, 485-499.
- European Commission** (2007). Commission recommendations of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes. Annex II to European Council Directive 86/609. See 2007/526/EC. Retrieved from <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=14OJ:L:2007:197:0001:0089:EN:PDF>.
- Facchin, L., Bisazza, A. and Vallortigara, G.** (1999). What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behav. Brain Res.* **103**, 229-234.
- Fitzgibbon, C. D.** (1990). Anti-predator strategies of immature Thomson's gazelles: hiding and the prone response. *Anim. Behav.* **40**, 846-855.
- Fotowat, H., Fayyazuddin, A., Bellen, H. J. and Gabbiani, F.** (2009). A novel neuronal pathway for visually guided escape in *Drosophila melanogaster*. *J. Neurophysiol.* **102**, 875-885.
- Fotowat, H., Harrison, R. R. and Gabbiani, F.** (2011). Multiplexing of motor information in the discharge of a collision detecting neuron during escape behaviors. *Neuron* **69**, 147-158.
- Frasnelli, E.** (2013). Brain and behavioral lateralization in invertebrates. *Front. Psychol.* **4**, 939.
- Frasnelli, E., Iakovlev, I. and Reznikova, Z.** (2012). Asymmetry in antennal contacts during trophallaxis in ants. *Behav. Brain Res.* **232**, 7-12.
- Geist, V.** (1971). *Mountain sheep. A study in Behavior and Evolution*. Chicago: University of Chicago Press.
- Ghirlanda, S. and Vallortigara, G.** (2004). The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proc. R. Soc. B Biol. Sci.* **271**, 853.
- Gillett, S. D. and Gonta, E.** (1978). Locust as prey: factors affecting their vulnerability to predation. *Anim. Behav.* **26**, 282-289.
- Graziano, M. S., Andersen, R. A. and Snowden, R. J.** (1994). Tuning of MST neurons to spiral motions. *J. Neurosci.* **14**, 54-67.
- Halloy, J., Sempo, G., Caprari, G., Rivault, C., Asadpour, M., Tâche, F., Saïd, I., Durier, V., Canonge, J., Amé, J. M. et al.** (2007). Social integration of robots into groups of cockroaches to control self-organized choices. *Science* **318**, 1155-1158.
- Halloy, J., Mondada, F., Kernbach, S. and Schmicke, T.** (2013). Towards bio-hybrid systems made of social animals and robots. In Conference on Biomimetic and Biohybrid Systems, pp. 384-386. Berlin, Heidelberg: Springer.
- Harcourt, J. L., Ang, T. Z., Sweetman, G., Johnstone, R. A. and Manica, A.** (2009). Social feedback and the emergence of leaders and followers. *Curr. Biol.* **19**, 248-252.
- Hawlena, D., Kress, H., Dufresne, E. R. and Schmitz, O. J.** (2011). Grasshoppers alter jumping biomechanics to enhance escape performance under chronic risk of spider predation. *Funct. Ecol.* **25**, 279-288.
- Herbert-Read, J. E., Krause, S., Morrell, L. J., Schaerf, T. M., Krause, J. and Ward, A. J. W.** (2012). The role of individuality in collective group movement. *Proc. R. Soc. B* **280**, 20122564.
- Homberg, U.** (2004). In search of the sky compass in the insect brain. *Naturwissenschaften* **91**, 199-208.
- Honma, A., Oku, S. and Nishida, T.** (2006). Adaptive significance of death feigning posture as a specialized inducible defence against gape-limited predators. *Proc. R. Soc. Lond. B Biol. Sci.* **273**, 1631-1636.
- Horridge, G. A.** (1962). Learning of leg position by headless insects. *Nature* **193**, 697-698.
- Horridge, G. A.** (1977). Insects which turn and look. *Endeavour* **1**, 7-17.
- Ijspeert, A. J., Crespi, A. and Cabelguen, J.-M.** (2005). Simulation and robotics studies of salamander locomotion. *Neuroinformatics* **3**, 171-195.
- Ingle, D.** (1973). Two visual systems in the frog. *Science* **181**, 1053-1055.
- Jolles, J. W., Boogert, N. J., Sridhar, V. H., Couzin, I. D. and Manica, A.** (2017). Consistent individual differences drive collective behavior and group functioning of schooling fish. *Curr. Biol.* **27**, 2862-2868.
- Karplus, I. and Algom, D.** (1981). Visual cues for predator face recognition by reef fishes. *Ethology* **55**, 343-364.
- Kim, C., Ruberto, T., Phamduy, P. and Porfiri, M.** (2018). Closed-loop control of zebrafish behaviour in three dimensions using a robotic stimulus. *Sci. Rep.* **8**, 657.
- Kral, K. and Poteser, M.** (1997). Motion parallax as a source of distance information in locusts and mantids. *J. Insect Behav.* **10**, 145-163.
- Kratović, L. and Frynta, D.** (2002). Body size, male combat and the evolution of sexual dimorphism in eublepharid geckos (Squamata: Eublepharidae). *Biol. J. Linn. Soc.* **76**, 303-314.
- Krause, J., Winfield, A. F. T. and Deneubourg, J.-L.** (2011). Interactive robots in experimental biology. *Trends Ecol. Evol.* **26**, 369-375.
- Kurylas, A. E., Rohlfing, T., Kroficzek, S., Jenett, A. and Homberg, U.** (2008). Standardized atlas of the brain of the desert locust, *Schistocerca gregaria*. *Cell Tissue Res.* **333**, 125.
- Kurzina, N., Aristova, I. and Volnova, A.** (2018). Lateralization of motor reactions and formation of behavioural tactics during learning in the eight-arm radial maze in adolescent and adult rats. *Laterality* **23**, 101-112.
- Li, F., Liu, W., Fu, X., Bonsignori, G., Scarfogliero, U., Stefanini, C. and Dario, P.** (2012). Jumping like an insect: Design and dynamic optimization of a jumping mini robot based on bio-mimetic inspiration. *Mechatronics* **22**, 167-176.
- Lippolis, G., Bisazza, A., Rogers, L. J. and Vallortigara, G.** (2002). Lateralisation of predator avoidance responses in three species of toads. *Laterality* **7**, 163-183.
- Lippolis, G., Westman, W., McAllan, B. and Rogers, L.** (2005). Lateralisation of escape responses in the stripe-faced dunnart, *Sminthopsis macroura* (Dasyuridae: Marsupialia). *Laterality* **10**, 457-470.
- Lippolis, G., Joss, J. M. P. and Rogers, L. J.** (2009). Australian lungfish (*Neoceratodus forsteri*): a missing link in the evolution of complementary side biases for predator avoidance and prey capture. *Brain Behav. Evol.* **73**, 295-303.
- Liu, G., Seiler, H., Wen, A., Zars, T., Ito, K., Wolf, R., Heisenberg, M. and Liu, L.** (2006). Distinct memory traces for two visual features in the *Drosophila* brain. *Nature* **439**, 551.
- Merzenich, M. M.** (2001). Cortical plasticity contributing to child development. In *Mechanisms in Cognitive Development* (ed. J. McClelland and R. Siegler), pp. 67-96. Mahwah, NJ: Erlbaum.
- Millon, A. and Bretagnolle, V.** (2008). Predator population dynamics under a cyclic prey regime: numerical responses, demographic parameters and growth rates. *Oikos* **117**, 1500-1510.
- Oliva, D., Medan, V. and Tomsic, D.** (2007). Escape behavior and neuronal responses to looming stimuli in the crab *Chasmagnathus granulatus* (Decapoda: Grapsidae). *J. Exp. Biol.* **210**, 865-880.
- O'shea, M., Rowell, C. H. F. and Williams, J. L. D.** (1974). The anatomy of a locust visual interneurone; the descending contralateral movement detector. *J. Exp. Biol.* **60**, 1-12.
- Partan, S. R., Larco, C. P. and Owens, M. J.** (2009). Wild tree squirrels respond with multisensory enhancement to conspecific robot alarm behaviour. *Anim. Behav.* **77**, 1127-1135.
- Peron, S. and Gabbiani, F.** (2009). Spike frequency adaptation mediates looming stimulus selectivity in a collision-detecting neuron. *Nat. Neurosci.* **12**, 318.
- Polverino, G., Abaid, N., Kopman, V., Macri, S. and Porfiri, M.** (2012). Zebrafish response to robotic fish: preference experiments on isolated individuals and small shoals. *Bioinspir. Biomim.* **7**, 036019.
- Preuss, T., Osei-Bonsu, P. E., Weiss, S. A., Wang, C. and Faber, D. S.** (2006). Neural representation of object approach in a decision-making motor circuit. *J. Neurosci.* **26**, 3454-3464.
- Rigosi, E., Haase, A., Rath, L., Anfora, G., Vallortigara, G. and Szyszka, P.** (2015). Asymmetric neural coding revealed by in vivo calcium imaging in the honey bee brain. *Proc. R. Soc. Lond. B Biol. Sci.* **282**, 20142571.
- Rind, F. C.** (2002). Motion detectors in the locust visual system: from biology to robot sensors. *Micros. Res. Techniq.* **56**, 256-269.
- Rodríguez-Romaguera, J. and Stuber, G. D.** (2018). Social isolation co-opts fear and aggression circuits. *Cell* **173**, 1071-1072.
- Rogers, L. J. and Vallortigara, G.** (2008). From antenna to antenna: lateral shift of olfactory memory recall by honeybees. *PLoS ONE* **3**, e2340.
- Rogers, L. J. and Vallortigara, G.** (2015). When and why did brains break symmetry? *Symmetry* **7**, 2181-2194.



- Rogers, L. J., Vallortigara, G. and Andrew, R. J. (2013a). *Divided Brains: the Biology and Behaviour of Brain Asymmetries*. Cambridge: Cambridge University Press.
- Rogers, L. J., Rigosi, E., Frasnelli, E. and Vallortigara, G. (2013b). A right antenna for social behaviour in honeybees. *Sci. Rep.* **3**, 2045.
- Rogers, L. J., Frasnelli, E. and Versace, E. (2016). Lateralized antennal control of aggression and sex differences in red mason bees, *Osmia bicornis*. *Sci. Rep.* **6**, 29411.
- Romano, D., Canale, A. and Benelli, G. (2015). Do right-biased boxers do it better? Population-level asymmetry of aggressive displays enhances fighting success in blowflies. *Behav. Processes* **113**, 159-162.
- Romano, D., Kavallieratos, N. G., Athanassiou, C. G., Stefanini, C., Canale, A. and Benelli, G. (2016a). Impact of geographical origin and rearing medium on mating success and lateralization in the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *J. Stored Prod. Res.* **69**, 106-112.
- Romano, D., Donati, E., Canale, A., Messing, R. H., Benelli, G. and Stefanini, C. (2016b). Lateralized courtship in a parasitic wasp. *Laterality* **21**, 243-254.
- Romano, D., Benelli, G. and Stefanini, C. (2017a). Escape and surveillance asymmetries in locusts exposed to a Guinea fowl-mimicking robot predator. *Sci. Rep.* **7**, 12825.
- Romano, D., Benelli, G., Donati, E., Remorini, D., Canale, A. and Stefanini, C. (2017b). Multiple cues produced by a robotic fish modulate aggressive behaviour in Siamese fighting fishes. *Sci. Rep.* **7**, 4667.
- Romano, D., Benelli, G., Stefanini, C., Desneux, N., Ramirez-Romero, R., Canale, A. and Lucchi, A. (2018a). Behavioral asymmetries in the mealybug parasitoid *Anagyrus* sp. near *pseudococci*: does lateralized antennal tapping predict male mating success? *J. Pest Sci.* **91**, 341-349.
- Romano, D., Donati, E., Benelli, G., and Stefanini, C. (2018b). A review of animal-robot interaction: from bio-hybrid organisms to mixed societies. *Biol. Cybern.* 1-25.
- Rowell, C. F. (1961). The structure and function of the prothoracic spine of the desert locust, *Schistocerca gregaria* Forskål. *J. Exp. Biol.* **38**, 457-469.
- Rowell, C. F. (1964). Central control of an insect segmental reflex: I. Inhibition by different parts of the central nervous system. *J. Exp. Biol.* **41**, 559-572.
- Ruxton, G. (2006). Behavioural ecology: Grasshoppers don't play possum. *Nature* **440**, 880.
- Ruxton, G. D., Sherratt, T. N. and Speed, M. P. (2004). *Avoiding Attack: the Evolutionary Ecology of Crypsis, Warning Signals and Mimicry*. Oxford: Oxford University Press.
- Santer, R. D., Simmons, P. J. and Rind, F. C. (2005). Gliding behaviour elicited by lateral looming stimuli in flying locusts. *J. Comp. Physiol. A* **191**, 61-73.
- Santer, R. D., Rind, F. C., Stafford, R. and Simmons, P. J. (2006). Role of an identified looming-sensitive neuron in triggering a flying locust's escape. *J. Neurophysiol.* **95**, 3391-3400.
- Santer, R. D., Yamawaki, Y., Rind, F. C. and Simmons, P. J. (2008). Preparing for escape: an examination of the role of the DCMD neuron in locust escape jumps. *J. Comp. Physiol. A* **194**, 69-77.
- Schlottterer, G. R. (1977). Response of the locust descending movement detector neuron to rapidly approaching and withdrawing visual stimuli. *Can. J. Zool.* **55**, 1372-1376.
- Seghier, M. L. (2008). Laterality index in functional MRI: methodological issues. *Magn. Reson. Imaging* **26**, 594-601.
- Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature* **138**, 32.
- Shtyrov, Y., Nikulin, V. V. and Pulvermüller, F. (2010). Rapid cortical plasticity underlying novel word learning. *J. Neurosci.* **30**, 16864-16867.
- Simmons, P. (1980). Connexions between a movement-detecting visual interneurone and flight motoneurons of a locust. *J. Exp. Biol.* **86**, 87-97.
- Stefanini, C., Orofino, S., Manfredi, L., Mintchev, S., Marrazza, S., Assaf, T., Capantini, L., Sinibaldi, E., Grillner, S., Wallén, P. et al. (2012). A novel autonomous, bioinspired swimming robot developed by neuroscientists and bioengineers. *Bioinspir. Biomim.* **7**, 025001.
- Strausfeld, N. J. (2005). The evolution of crustacean and insect optic lobes and the origins of chiasmata. *Arthropod Struct. Dev.* **34**, 235-256.
- Strauss, R. (2002). The central complex and the genetic dissection of locomotor behaviour. *Curr. Opin. Neurobiol.* **12**, 633-638.
- Strauss, R. and Heisenberg, M. (1993). A higher control center of locomotor behavior in the *Drosophila* brain. *J. Neurosci.* **13**, 1852-1861.
- Thorogood, J. and Whimster, I. W. (1979). The maintenance and breeding of the Leopard gecko. *Int. Zoo Yearb.* **19**, 74-78.
- Tinbergen, N. (1951). *The Study of Instinct*. Oxford University Press.
- Todd, D. J. (1993). Mobile robots—the lessons from nature. In *Robots and biological systems: towards a new bionics?*, pp. 193-206. Berlin, Heidelberg: Springer.
- Vallortigara, G. and Rogers, L. J. (2005). Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav. Brain Sci.* **28**, 575-588.
- Vallortigara, G. and Versace, E. (2017). Laterality at the neural, cognitive, and behavioral levels. In *APA handbook of Comparative Psychology: vol. 1. Basic Concepts, Methods, Neural Substrate, and Behavior* (ed. J. Call), pp. 557-577. Washington DC: American Psychological Association.
- Vallortigara, G., Chiandetti, C. and Sovrano, V. A. (2011). Brain asymmetry (animal). *Wiley Interdiscip. Rev. Cogn. Sci.* **2**, 146-157.
- Wang, Y. and Frost, B. J. (1992). Time to collision is signaled by neurons in the nucleus rotundus of pigeons. *Nature* **356**, 236.
- Webb, B. (1995). Using robots to model animals: a cricket test. *Robot. Auton. Syst.* **16**, 117-134.
- Webb, B. (2000). What does robotics offer animal behaviour? *Anim. Behav.* **60**, 545-558.
- Wicklein, M. and Strausfeld, N. J. (2000). Organization and significance of neurons that detect change of visual depth in the hawk moth *Manduca sexta*. *J. Comp. Neurol.* **424**, 356-376.
- Woo, K. L. and Rieucau, G. (2011). From dummies to animations: a review of computer-animated stimuli used in animal behavior studies. *Behav. Ecol. Sociobiol.* **65**, 1671.
- Wood, R. J. (2008). The first takeoff of a biologically inspired at-scale robotic insect. *IEEE T. Robot.* **24**, 341-347.
- Yamamoto, K., Nakata, M. and Nakagawa, H. (2003). Input and output characteristics of collision avoidance behavior in the frog *Rana catesbeiana*. *Brain Behav. Evolut.* **62**, 201-211.
- Ydenberg, R. C. and Dill, L. M. (1986). The economics of fleeing from predators. In *Advances in the Study of Behavior*, vol. **16**, pp. 229-249. Academic Press.

**Table S1.** Lateralized parameters characterizing the jumping escape response of different trained *Locusta migratoria* during the symmetric exposure to the robotic leopard gecko (values are means±SE).

LATERALIZED PARAMETER	NA	LA	RA	NIV	LIV	RIV	NII	LII	RII
LI of the jumping escape response	-0.06 ± 0.15	0.35 ± 0.09	-0.50 ± 0.07	-0.03 ± 0.09	0.55 ± 0.08	-0.65 ± 0.06	-0.04 ± 0.03	0.66 ± 0.07	-0.83 ± 0.03
ABLI of the escape response	0.70 ± 0.04	0.55 ± 0.04	0.58 ± 0.04	0.37 ± 0.04	0.65 ± 0.04	0.69 ± 0.04	0.13 ± 0.02	0.72 ± 0.04	0.83 ± 0.03
number of right-biased jumps	14.16 ± 2.2	20.24 ± 1.42	7.56 ± 1.07	14.4 ± 1.28	23.32 ± 1.2	5.28 ± 0.9	14.48 ± 0.52	24.96 ± 1.02	2.6 ± 0.5
number of left-biased jumps	15.84 ± 2.20	9.76 ± 1.42	22.44 ± 1.08	15.6 ± 1.29	6.68 ± 1.20	24.72 ± 0.91	15.56 ± 0.52	5.04 ± 1.03	27.4 ± 0.51
number of right-biased subjects	12	20	2	9	22	1	2	24	0
number of left-biased subjects	13	5	23	10	3	24	1	1	25

LI = laterality index; ABLI = absolute value of the laterality index; NA = Naïve adult; LA = Left trained adult; RA = Right trained adult; NIV = Naïve IV instar; LIV = Left trained IV instar; RIV = Right trained IV instar; NII = Naïve II instar; LII = Left trained II instar; RII = Right trained II instar.

**Table S2.** Lateralized parameters characterizing surveillance of different trained *Locusta migratoria* during the symmetric exposure to the robotic leopard gecko (values are means±SE).

LATERALIZED PARAMETER	NA	LA	RA	NIV	LIV	RIV	NII	LII	RII
LI of the surveillance response	0.62 ± 0.10	0.56 ± 0.10	0.65 ± 0.11	0.58 ± 0.10	0.41 ± 0.12	0.64 ± 0.09	0.46 ± 0.12	0.41 ± 0.11	0.67 ± 0.10
ABLI of the surveillance response	0.79 ± 0.03	0.71 ± 0.03	0.83 ± 0.02	0.74 ± 0.03	0.70 ± 0.03	0.76 ± 0.04	0.74 ± 0.03	0.65 ± 0.04	0.82 ± 0.04
Right eye use during surveillance (min)	22.52 ± 1.49	19.48 ± 1.25	22.44 ± 1.54	20.24 ± 1.34	18.08±1.54	22.56 ± 1.30	18.92 ± 1.65	18.36 ± 1.45	22.96 ± 1.48
Left eye use during surveillance (min)	5 ± 1.30	5.48 ± 1.18	4.24 ± 1.17	5.36 ± 1.26	7.24 ± 1.39	4.68 ± 1.08	6.68 ± 1.48	7.68 ± 1.41	4.52 ± 1.39
number of right-biased subjects	22	22	22	22	20	23	20	20	22
number of left-biased subjects	3	3	3	3	5	2	5	5	3

LI = laterality index; ABLI = absolute value of the laterality index; NA = Naïve adult; LA = Left trained adult; RA = Right trained adult; NIV = Naïve IV instar; LIV = Left trained IV instar; RIV = Right trained IV instar; NII = Naïve II instar; LII = Left trained II instar; RII = Right trained II instar.