Adaptive decision-making by ants in response to past, imminent, and predicted adversity

AUTHOR

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KEY WORDS

Visual orientation, Swimming, Locomotor pattern, Substrate condition, Internal state, Scototaxis

ABSTRACT

Many animals exhibit innate behaviours, which are often interpreted as hard-wired, reflex-like responses, particularly in insects. Among these behaviours, beacon-aiming—an approach towards dark areas or objects—is observed in many animals; however, its functional significance remains unclear, and some ant species do not exhibit it. Here we show that in one such species, *Camponotus japonicus,* the behaviour was triggered only under adverse substrate conditions, such as during the crossing of liquid-covered surfaces, regardless of the locomotor patterns like walking or swimming, or the mere presence of water, or when walking upside-down. Once initiated, beacon-aiming persists even under normal substrate conditions, as demonstrated by ants transitioning from water-covered to dry substrates suitable for comfortable walking. This behavioural flexibility indicates that the innate behaviour is not hard-wired but modulated by internal states. Furthermore, changes in internal states may serve adaptive decision-making, potentially allowing ants to prepare for future adverse conditions. The isolated ants on a water-surrounded platform gradually established an attraction to the direction of the beacon before ultimately swimming towards it. These findings suggest that beacon-aiming is regulated by internal states, especially anxiety-like states formed in response to past, imminent, and predicted adverse substrate conditions in ants.

1. INTRODUCTION

Decision-making processes in animals are highly intricate, providing flexible behavioural outputs that adapt to environmental and behavioural contexts [1,2]. While larger brains enable advanced cognitive processing, they also bring about significant metabolic costs arising from development and operation [3,4]. Importantly, cognitive function is not strictly dependent on brain size [5]. Even insects with small brains and limited computational resources [6,7] are capable of complex cognitive processing [8,9], such as path integration [10,11], tool use

(reviewed in [12]), and multi-modal cue integration [13]. Such processes are often governed by learning, which involves plasticity in neural circuits [14]. Learning allows some forms of inference, even in insects [15,16]. Additionally, insect behaviour can be temporarily promoted or suppressed by physiological factors, such as the perception of threats during courtship [17] and winner and loser effects [18].

Such transient behavioural changes are not limited to learned actions but also occur in innate behaviours [19,20], which are typically considered relatively fixed [21]. However, the neural mechanisms underlying the dynamic modulation of innate behaviours remain poorly understood [22,23]. This gap partly arises from the paucity of simple experimental paradigms to manipulate decision-making processes involved in innate behaviours, which are defined by their stability and induction through simple stimuli [24,25].

In this study, we focus on beacon-aiming [26], a behaviour consisting of a spontaneous orientation towards visually conspicuous dark objects. In ethological contexts, this behaviour is classified as scototaxis and has been observed across almost all insect orders (reviewed in [27]) and other invertebrates [28,29]. Beacon-aiming has also been reported in insects that fall onto water surfaces [30,31]. While many species experience difficulties upon falling into water [31,32], some are capable of coordinated locomotion across water surfaces [33,34].

In ants, beacon-aiming on land or water has been documented in various species [35,36] and is typically interpreted as an innate response, independent of age, feeding state, or caste [27,37,38]. However, a comparative study found that some ant species do not exhibit beacon-aiming on plastic surfaces of experimental arenas [27].

In the present study, we departed from the hypothesis that poor substrate engagement impairing locomotion could be a trigger for beacon-aiming. Choosing a species that did not perform beacon-aiming in previous tests, the partially arboreal *Camponotus japonicus* [39] capable of climbing extremely smooth vertical walls, we show that it does perform beacon-

aiming when substrate conditions, such as immersion in water, are adverse. We then introduce experimental paradigms in which an ant has to transition from one substrate to another, i.e. from water to dry substrate and vice-versa. This set-up allows us to probe the decision-making dynamics in ants before, during, and after transition between substrates.

2. MATERIALS AND METHODS

(a) Animals

Foragers of the Japanese carpenter ant (*Camponotus japonicus* Mayr, 1866) were collected from Komaba I, II campus of the University of Tokyo (35.661°N, 139.684°E; 35.662°N, 139.678°E) and were individually isolated in 50 ml centrifuge tubes. Prior to the experiments, they were acclimatised under laboratory conditions with light exposure for at least one hour. Only physically intact (no visible damage to legs and antennae) ants were selected for the experiments.

(b) General procedures

All experiments were conducted inside an opaque cylindrical arena (radius, approximately 30 cm). A vertical black rectangle (beacon) measuring 20° horizontally and 45° vertically from the effective centre of the floor was placed on the inner wall of the arena, based on prior studies [27,40,41]. To minimise the potential influence of external cues, the beacon was placed in one of three possible positions, each separated by 120°, with trials evenly distributed across these positions [27]. Each trial began with the ant placed at the centre of the floor. The behavioural sequence was recorded using an ILME–FX3 camera (Sony) equipped with a 24–105mm F4 DG OS HSM lens (Sigma) at 59.94 fps, until the ants reached the edge of the circular area used for analysis (arena edge), as defined for each experiment.

94

95 **(c) Experimental procedures**

96 **(i) Influence of substrate conditions, locomotor patterns and water reception on beacon-**
97 **aiming**

98 To investigate whether *C. japonicus* exhibits different orientation responses on land and water
99 surfaces, two conditions were prepared: 'Land-walking' – walking on a dry substrate (figure 1a
100 corresponding to [27]); and 'Water-swimming' – forced swimming in the same arena filled with
101 water (figure 1b corresponding to [31]). To exclude the possibility that the observed behaviour
102 is specifically associated with locomotion unique to swimming, we provided the ants with a
103 'Water-wading' condition where a shallow water depth allowed the ants to walk (wade) using a
104 walking locomotor pattern (figure 1b). The behavioural sequence was recorded until the ant
105 reached a defined arena edge (radius, 20 cm).

106 Furthermore, the involvement of water reception in beacon-aiming was assessed by replacing
107 water with two kinds of liquids (figure 1c). 'Glycerol-swimming' involved swimming in a
108 mixture of 80% (v/v) glycerol and 20% (v/v) 2-propanol, which had a dynamic viscosity of
109 167.1 ± 2.1 cSt (average of three measurements at 24.0°C). In the 'Tylose-swimming' used a
110 1.0% (w/w) tylose (H 10000 P2, SE Tylose, Wiesbaden, Germany) aqueous solution adjusted
111 to closely match the dynamic viscosity of the glycerol-propanol mixture (166.5 ± 1.8 cSt,
112 average of three measurements at 23.7°C, respectively). A transparent glass Petri dish (radius,
113 10 cm), placed at the centre of the arena, was filled with liquid. The behavioural sequence was
114 recorded until the ant reached a defined arena edge (radius, 9 cm).

115 In addition, as an adverse substrate condition that does not employ liquids at all, we forced
116 ants to walk upside-down ('Upside-down-walking') on sandpaper (#2000). Under these
117 conditions, 32/77 ants dropped from the substrate, demonstrating it is difficult to negotiate for

them. The edge of the arena (radius, 9 cm) was defined in alignment with the dimensions of the sandpaper (23 cm × 28 cm).

(ii) After-effects of immersion after a water-to-land transition

Building on the results of experiment (i), we examined whether the directional decision persisted after a transition from water to a dry substrate (equivalent to aquathlon, figure 1d). The experiment was conducted under three conditions: 'Beacon Fixed' – the beacon remained stationary on the arena wall; 'Beacon Displaced' – the beacon was rapidly shifted by 60° upon the transition of the ants from water to dry substrate; and 'Walking-without-transition' – ants walked on a dry arena without a pool to account for differences in floor height and texture compared to the standard arena used in experiment (i). The behavioural sequence was recorded until they reached a defined arena edge (radius, 20 cm), passing through a defined pool edge (radius, 8 cm).

(iii) Decision-making process for beacon-aiming before a land-to-water transition

In preliminary experiments, we observed that workers of *C. japonicus*, once placed on a small, dry platform surrounded by water (referred to as an 'island'), spontaneously left the island by swimming after a period of exploration on the platform (which would correspond to reverse aquathlon). Leveraging this behaviour, we investigated whether the ants orient towards a beacon prior to starting to swim, thereby examining whether the decision-making process occurs independently of immersion.

A transparent glass cup was inverted to create an island (radius, 2.75 cm) at the centre of the arena, surrounded by water. To prevent ants from inadvertently leaving the island due to their

initial momentum, they were first confined within a Fluon-coated cylinder on the island. The cylinder was removed to begin the trial.

The behavioural sequence was classified into four distinct phases:

1. Initial Phase (prior to first water contact): This phase lasted until the mandibles crossed the defined edge of the island (radius, 2.75 cm).

2. Contact Phase (before first immersion, after first water contact): Starting at the end of the Initial Phase, this phase concluded when the mandibles crossed the defined immersion edge, represented by a circle with a radius equal to the island's radius plus two-thirds of the ant's body length. In this phase, ants contact water mainly with their antennae and forelegs during exploration (defined as water contacts).

3. Immersion Phase (before initiating swimming, after first immersion): This phase spanned from the end of the Contact Phase to the moment when the ant lost mechanical contact with the island. In this phase, ants protrude much more over the boundary, apparently exploring beyond for invisible gaps. This is defined as water immersion, but water contacts continue also during this phase.

4. Swimming Phase: Beginning at the end of the Immersion Phase, this phase ended when the ant reached the defined arena edge (radius, 20 cm).

(d) Analysis

Trajectories of the ants were obtained using DeepLabCut 2.3.8 [42], except for Upside-down-walking, where UMATracker Release-15 [43] was instead used. The direction of the vector connecting the centre of the arena to the position where the ant reached the arena edge was defined as the final bearing of each trial. Similarly, the final bearings at other defined edges and the direction of the body axis were also calculated.

Statistical analyses were conducted as previously described [27] using Python (https://www.python.org) and the package 'circular' (version: 0.4–93) implemented in R [44]. Circular box-and-whisker plots were generated using a modified version of R code from Buttarazzi et al (2018 [45]). The Rayleigh test was used to test for uniformity of circular distributions. Differences in circular distributions among experimental groups were evaluated using the Mardia–Watson–Wheeler test. For comparisons made for non-circular data groups, the Mann–Whitney U test was used. All statistical tests were two-tailed and Bonferroni correction applied where necessary.

Further details of MATERIALS AND METHODS are provided in the electronic supplementary material, text S1.

3. RESULTS

(a) Experiment (i): Influence of substrate conditions, locomotor patterns and water reception on beacon-aiming

Under 'Land-walking' conditions, ant trajectories were relatively straight (figure 2, electronic supplementary material, figure S1); however, their orientation appeared to be in random directions (figure 2a). This was reflected by a wide interquartile range spanning more than 90 degrees in the circular boxplot of the final bearings, indicating no clear directional preference (Rayleigh test: $\overline{R} = 0.19$, $P = 0.140$, $N = 57$).

Under 'Water-swimming' conditions, except for occasional difficulties, ants adopted a distinctive swimming posture with their antennae raised above the water and a locomotor pattern characterised by extended hind legs. During swimming, most trajectories meandered slightly but generally oriented towards the beacon or its vicinity (figure 2b). The interquartile range of the circular boxplot of the final bearings of Water-swimming conditions overlapped

with the beacon range and was relatively narrow, indicating a preference for the centre of the beacon (Rayleigh test: $\overline{R}$ = 0.95, $P < 0.001$, $N = 57$; mean direction: 1.3°, 95% confidence interval: [−1.8°, 4.4°]). The final bearing distribution was significantly different from Land-walking (Mardia–Watson–Wheeler test: $W_{(2)} = 73.01$, $P < 0.001$).

Under 'Water-wading' conditions, the distribution of final bearings (Rayleigh test: $\overline{R}$ = 0.97, $P < 0.001$, $N = 57$; mean direction: −2.1°, 95% confidence interval: [−5.9°, 1.6°], figure 2c) was similar to that in Water-swimming. The final bearing distribution was significantly different from Land-walking (Mardia–Watson–Wheeler test: $W_{(2)} = 69.28$, $P < 0.001$) but not significantly different from Water-swimming (Mardia–Watson–Wheeler test: $W_{(2)} = 3.12$, $P = 0.629$).

When introduced into a glycerol-propanol mixture or an aqueous solution of tylose, the smooth paddling motion of ants was hindered by the high viscosity of these liquids. Under 'Glycerol-swimming' conditions, they swam towards the beacon across the liquid surface, although their locomotion was impaired (Glycerol-swimming, Rayleigh test: $\overline{R}$ = 0.95, $P < 0.001$, $N = 37$; mean direction: 1.5°, confidence interval: [−4.2°, 6.9°], figure 2d). Under 'Tylose-swimming' conditions, ants also swam towards the beacon (Rayleigh test: $\overline{R}$ = 0.96, $P < 0.001$, $N = 49$; mean direction: 1.0°, confidence interval: [−3.3°, 5.2°], figure 2e). No significant difference was observed in the distribution of final bearings between swimming across these two liquids (Mardia–Watson–Wheeler test: Glycerol-swimming and Tylose-swimming: $W_{(2)} = 2.66$, $P = 0.265$). These results indicate that the presence of water is not required for beacon-aiming.

In addition, ants forced to perform upside-down walking on sandpaper also significantly oriented towards the beacon direction ('Upside-down-walking', Rayleigh test: $\overline{R}$ = 0.72, $P < 0.001$, $N = 45$; mean direction: 10.0°, confidence interval: [−2.7°, 24.1°], figure 2f). Therefore,

liquid immersion is not necessary to induce beacon-aiming, but it is considerably more convenient in experimental situations.

(b) Experiment (ii): Persistence of beacon-aiming decision made after water-to-land transition

Swimming in a pool placed at the centre of the arena induced beacon-aiming, shown as the distributions of the final bearings on the pool edge under all conditions ('Beacon Fixed', 'Beacon Displaced': Rayleigh test: $\overline{R}$ = 0.85, 0.84, both $P < 0.001$, $N = 33, 33$, mean direction: 1.0°, 0.0°, confidence interval: [−8.2°, 9.2°], [−7.9°, 7.3°], figure 3b, c, respectively).

Under Beacon Fixed conditions, beacon-aiming persisted after the transition from water to land (Rayleigh test: $\overline{R}$ = 0.78, $P < 0.001$, $N = 33$, mean direction: −2.7°, confidence interval: [−10.5°, 5.3°], figure 3d). No significant difference was observed in the distributions of final bearings at the pool edge and arena edge (Mardia–Watson–Wheeler test: $W_{(2)} = 0.27$, $P = 0.875$). Under 'Walking-without-transition' conditions on the same dry polystyrene substrate, no orientation towards the beacon was observed (Rayleigh test: $\overline{R}$ = 0.28, $P = 0.145$, $N = 33$, figure 3a, electronic supplementary material, figure S2). The distribution of final bearings at arena edge in the water to land transition experiment under Beacon Fixed conditions was significantly different from Walking-without-transition (Mardia–Watson–Wheeler test: $W_{(2)} = 25.74$, $P < 0.001$, $N = 33, 33$).

Under Beacon Displaced conditions, the beacon-aiming persisted and the directional decision was updated even after the transition from water to land, as indicated by the mean direction shifting towards the new beacon position, which was displaced by 60° (Rayleigh test: $\overline{R}$ = 0.61, $P < 0.01$, $N = 33$, mean direction: 31.5°, confidence interval: [12.1°, 48.6°], figure 3e). This shift resulted in a significant difference between the distributions of final bearings at the pool

edge and the arena edge (Watson−Wheeler test: $W_{(2)} = 30.58$, $P < 0.001$) as well as between the distributions of final bearings at arena edge under Beacon Fixed and Beacon Displaced conditions (Mardia–Watson–Wheeler test: $W_{(2)} = 30.69$, $P < 0.001$). Final bearings in the Beacon Displaced condition exhibited a somewhat variable partial shift to the new beacon position such that no significant difference was found in comparison to the Walking-without-transition condition (Mardia–Watson–Wheeler test: $W_{(2)} = 7.72$, $P = 0.021$, $N = 33, 33$, with the Bonferroni correction accounted for multiple comparisons among three conditions). However, the body axis direction was significantly oriented towards the new beacon position (Rayleigh test: $\overline{R} = 0.50$, $P < 0.001$, mean direction: 68.6°, confidence interval: [45.1°, 92.7°], figure 3f). Once the ants reached the shore, they did not re-enter the water.

(c) Experiment (iii): Decision-making process of beacon-aiming before a land-to-water transition

Ants, after released onto an island surrounded by water, showed distinct phases of behaviour. During the 'Initial Phase', the ants contacted water without a directional bias (Rayleigh test: $\overline{R} = 0.05$, $P = 0.917$, $N = 33$, figure 4a, b). However, a significant directional bias was evident in the distribution of all water contacts during the 'Contact Phase' (Rayleigh test: $\overline{R} = 0.27$, $P < 0.001$, $N = 282$, mean direction: 23.2°, confidence interval: [6.3°, 39.8°], figure 4a, c, d, electronic supplementary material, figure S3a), which is interpreted as a preference for the right side of the beacon (10°). Similarly, the distribution of the first immersions is significantly biased to the right edge of the beacon (Rayleigh test: $\overline{R} = 0.34$, $P = 0.033$, $N = 33$, mean direction: 6.0°, confidence interval: [−33.5°, 53.0°], figure 4c), and the distribution of all water immersions is also significantly biased to the right edge of the beacon (Rayleigh test: $\overline{R} = 0.66$, $P < 0.001$, $N = 33$, mean direction: 13.2°, confidence interval: [10.5°, 15.7°], figure 4a, e, f).

After several water immersions (electronic supplementary material, figure S3b), the ants exhibited 'pre-swimming behaviour'. Some behaviours classified as immersions were identified as pre-swimming behaviour. This behaviour, characterised by paddling movements while maintaining contact with the shore using at least one hind leg, displayed intermediate characteristics between walking and swimming, complicating quantitative analysis. Most ants began swimming after clear pre-swimming behaviour, showing a directional bias towards the beacon (Rayleigh test: $\overline{R}$ = 0.94, $P < 0.001$, $N = 33$, mean direction: 7.0°, confidence interval: [6.1°, 14.8°], figure 4a, e). During the 'Swimming Phase', ants finally decided orienting towards the centre of the beacon (Rayleigh test: $\overline{R}$ = 0.99, P < 0.001, $N = 33$, mean direction: 1.4°, confidence interval: [-1.0°/3.9°], figure 4a, g).

These results suggest that while the ants showed no interest in the beacon during the Initial Phase, they exhibited a preference for the sides or edges of the beacon during the Contact Phase, eventually aligning themselves with the centre of the beacon after swimming.

4. Discussion

Animals act autonomously in diverse and complex natural environments. The elements defining their behaviour have been classified into 1) reflexes, fixed action patterns, and innate behaviours which can be seen as characteristics of an automatic machine, the extensions of such a machine, or a combination of both, and 2) learning, decision-making, and related processes which are key elements enabling flexible adaptation to diverse natural environments. The primary focus of the research aimed at understanding flexibility and adaptability has been on the latter.

While some ant species are known to orient towards a visual landmark on land [26,35,37], recent findings have revealed that *C. japonicus* does not exhibit such orientation under

terrestrial conditions [27], except in cases where the landmark is associated with appetitive learning [46]. Our research showed that the attraction to the beacon in this species when crossing water surfaces was triggered by immersion rather than by reception of water itself or the use of swimming-specific locomotor patterns (experiment (i)). Changes in orientation decisions depending on substrate conditions are known from a few intertidal species in specific ecological contexts [47,48]. When forced to walk upside-down under danger of dropping, the ants performed beacon-aiming too. Insects are known to internally monitor their walking movements, detecting reductions in walking speed and/or abnormal leg rotation rates [49]. Therefore, they are sensitive to the state of contact with the substrate. This is especially true for arboreal arthropods, as dropping from arboreal environments can induce isolation from familiar environments and/or falling onto water surfaces. Unfamiliar environments pose difficulties for navigation, which is a significant problem, particularly for central place foragers [50,51]. Furthermore, movements across water surfaces pose risks of drowning or predation, especially for small animals [52–54]. Thus, adverse substrate conditions or even adverse conditions in general are candidates for inducing beacon-aiming. This may apply to other arboreal arthropods or even a multitude of terrestrial animals.

Context-dependent decision-making has been studied in diverse animal species and in various contexts [55,56]. Although innate behaviours are generally considered to be hard-wired [37,57], a certain degree of context-dependence has been reported [19,58]. However, it remains to be explained whether these context-dependent responses are similar to program behaviours triggered directly by a specific sensory input [59] or are instead regulated by transient changes in internal state [60,61].

Ants having exhibited beacon-aiming in the pool (experiment (ii)) continued to orient towards the beacon even after beginning to walk. This result contrasts with the absence of beacon-aiming without prior swimming experience. Most ants re-oriented themselves towards

310 the displaced beacon, suggesting that their behaviour was not merely maintaining directional
311 consistency or a direct sensory behaviour; rather, it should be regarded as a continuation of an
312 internal state [23] that induces beacon-aiming. Internal states provide specialized consistency
313 to behavioural tendencies in animals across similar behavioural contexts, such as hunger [62],
314 thirst [63], social isolation [64], and focusing on courtship [17]. The temporal persistence of
315 internal states has been observed in other arthropods [65,66].

316 Ants having been introduced onto the island (experiment (iii)) made initial contacts with the
317 surrounding water in various directions and then began to access boundary areas in different
318 directions. Thorough scanning in unfamiliar visual environments in ants aligns with previous
319 observations [67]. It is noteworthy that the orientation of the ants towards the periphery of the
320 beacon was induced prior to the experience of immersion. Furthermore, even after repeated
321 water contacts and subsequent beacon-aiming, the ants often continued to explore other
322 directions thoroughly (electronic supplementary material, figure S3b). This observation
323 suggests that beacon-aiming in ants is not a binary response but rather a behaviour shaped
324 gradually and most likely caused by the formation of an anxiety-like state [68]. In summary,
325 the results of our study demonstrate that beacon-aiming in the ant *C. japonicus* elicited by
326 adverse substrate conditions can be interpreted as an anxiety-like behaviour.

327 Anxiety-like behaviour has been studied primarily in rodents [69] and fish [70], as well as in
328 some arthropods [71,72], including insects [73], yet detailed research remains scarce for non-
329 model species. The dynamic changes in orientation decisions observed in *C. japonicus* on an
330 island, which might be viewed as a reverse analogue of the Morris water maze, illustrate the
331 process of anxiety-like state formation. To the best of our knowledge, spontaneous escape
332 behaviours from a platform have been studied only in a few invertebrates [74,75].

333 A commonly used experimental paradigm in studies of anxiety is the light-dark test, which
334 induces a preference for darkness (scototaxis) in animals. The simplest explanation for the

ecological significance of this preference is that the bright, open areas are innately aversive to many animals, whereas darker spaces are relatively predictable as more secure under predation risks [76,77], making them valuable to approach or remain in. Scototaxis has been observed in rodents [78,79], fishes [80], insects [81,82], crustaceans [83,84], arachnids [85], echinoderms [86], and molluscs [29,87]. Conversely, this innate preference can be altered depending on habitat [88], developmental stage [89], and time of day [88].

The relationship between beacon-aiming and dark preference in general remains to be explained. For instance, tendencies such as preference for an area which includes a number of dark spots over overall darkness [22], back-and-forth movement between black stripes in Buridan's paradigm [90], and attraction to a bright beacon against a dark background during swimming [40] have been observed. If these behaviours are included in beacon-aiming, the relationship between scototaxis as an anxiety-like behaviour and beacon-aiming would have to be reconsidered.

Is beacon-aiming simply a result of an internal state leading to this behaviour or is there a more sophisticated cognitive process involved to decide to head for the beacon? This is something we cannot decide at this point. Interestingly, beacon-aiming on the island (experiment (iii)) is adapted to handle the adversity regardless of the actual mechanism that can be the result of a change in internal state [72] or cognitive processing [91]. Examples of responses to future adversity include ants avoiding similar situations by generalizing based on their experiences even after a single emergency encounter [92], and grasshoppers that, under chronic predation risk, learn to escape faster and farther [93].

Various substances are known to play a major role in forming and maintaining internal states [18,65], but knowledge about the mechanisms regulating innate behaviour remains limited [19,23]. Brain areas known to be involved in beacon-aiming are the central complex, which is necessary for turning during visual following in the context of beacon-aiming [94], and the

mushroom bodies, responsible for allowing navigation relative to a beacon by learning rather than aiming for it [95]. The activation of neurons expressing dNPF (*Drosophila* neuropeptide F) [96] or octopaminergic neurons [97] can induce beacon-aiming towards small objects that *Drosophila* otherwise avoid [41]. Whether such mechanisms are related to state changes that result in the behaviours we observe around transitions between substrates remains to be seen.

5. Conclusion

A simple and supposedly hard-wired orientation response, beacon-aiming, was shown to be induced by adverse substrate conditions in an ant species. Using paradigms with transitions between different substrates enabled us to observe dynamical aspects of the decisions for orientation. As both after-effects and pre-emptive effects could be shown, we conclude that substrate conditions adverse to locomotion are a factor altering the internal state of the ants, leading to an anxiety-like state which promotes the orientation towards beacons. Similarities to anxiety-like behaviours in other animals open up the interesting question whether similar behavioural strategies are used by a wide range of animal species.

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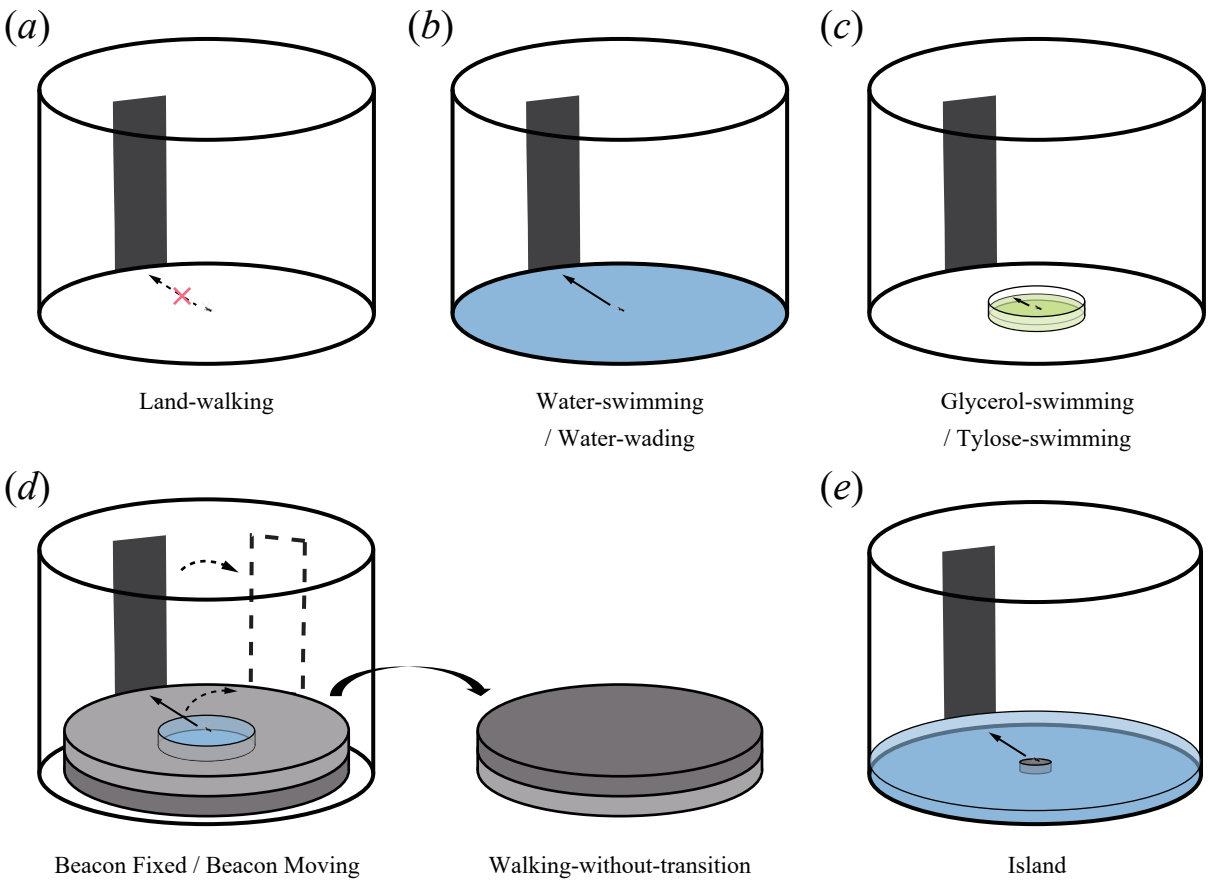

Figure 1. All experiments were conducted in an arena with a black beacon (height: 45°, width: 20°, measured from the effective centre of the floor) placed against the wall. Arrows and cross marks represent the overall orientation trend. (a) Basic arena used for Land-walking. (b) Arena filled with water for swimming (Water-swimming) or with shallow water for wading (Water-wading). (c) Arena, same as (a), with a transparent dish filled with either a 80% (v/v) glycerol and 20% (v/v) 2-propanol mixture (Glycerol-swimming) or a 1.0% (w/w) tylose aqueous solution (Tylose-swimming). (d) Arena fitted with extruded polystyrene foam insets to enable the transition from swimming in water to walking on a dry substrate. One side of the inset contained a central pool that could be filled with water (for the conditions Beacon Fixed, Beacon Displaced), while the other side served as a control (for condition Walking-without-transition), providing only the uniform dry substrate. The final position of the beacon, which was displaced by 60° under the Beacon Displaced condition, is also illustrated (dashed outline). (e) Arena, same as (b), with a transparent cup in the centre and the water level matched to the base of the cup (Island), effectively creating a central platform surrounded by water ("island").

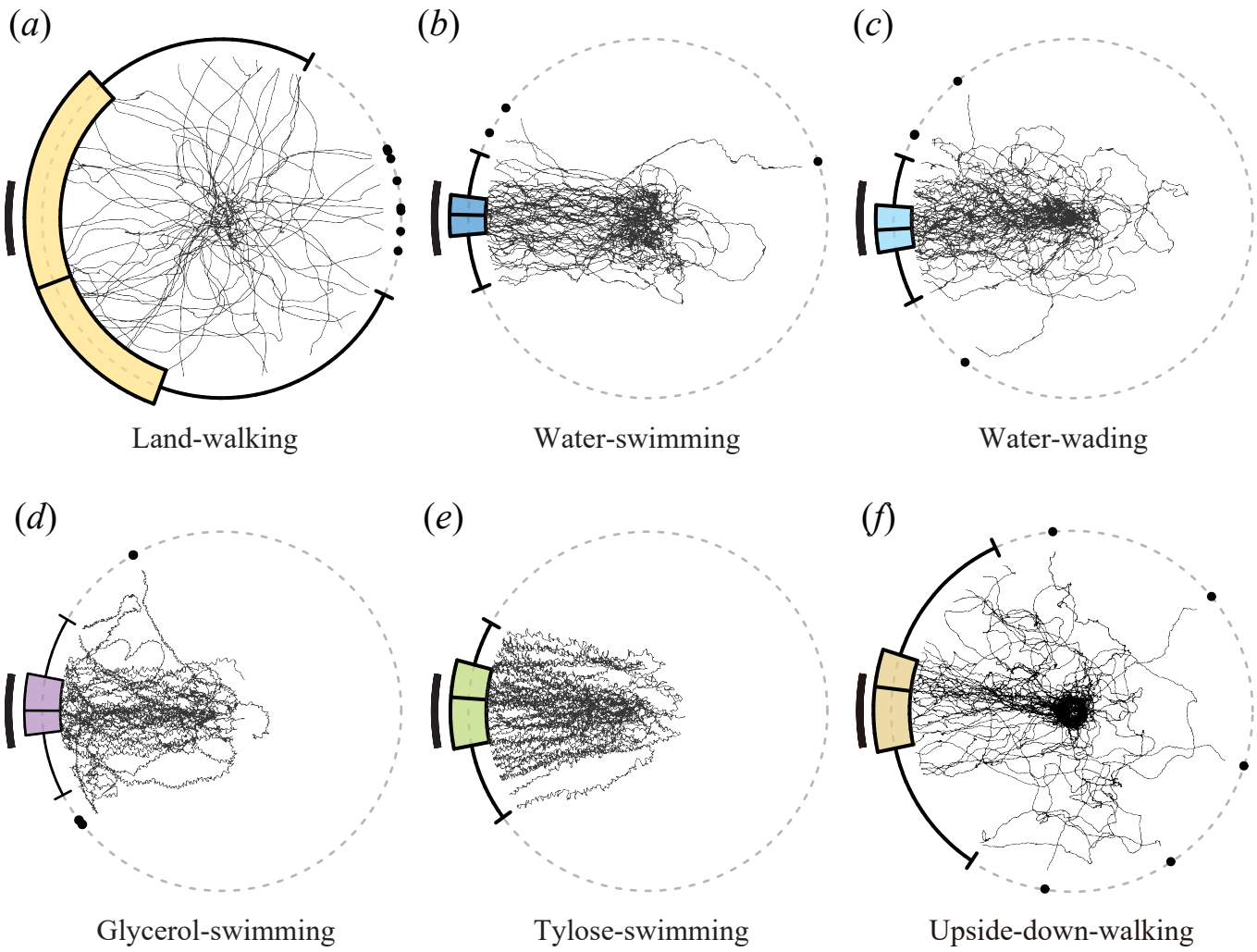

Figure 2 Trajectories and circular box-and-whisker plots of final bearings are shown under the following conditions: (a) Land-walking (walking on dry substrate, *N* = 57); (b) Water-swimming (swimming in water, *N* = 57); (c) Water-wading (walking in shallow water, *N* = 57); (d) Glycerol-swimming (swimming in an 80% (v/v) glycerol and 20% (v/v) 2-propanol mixture, *N* = 47); and (e) Tylose-swimming (swimming in a 1.0% (w/w) tylose aqueous solution, *N* = 59); (f) Upside-down-walking (*N* = 45). The beacon range is represented as a black arc segment on the left side of each plot. Individual trajectories correspond to single trials. Circular box-and-whisker plots show the median, first quartile, third quartile (coloured interquartile range), minimum, maximum (whiskers), and outliers (black-filled circles) of the final bearing distributions.

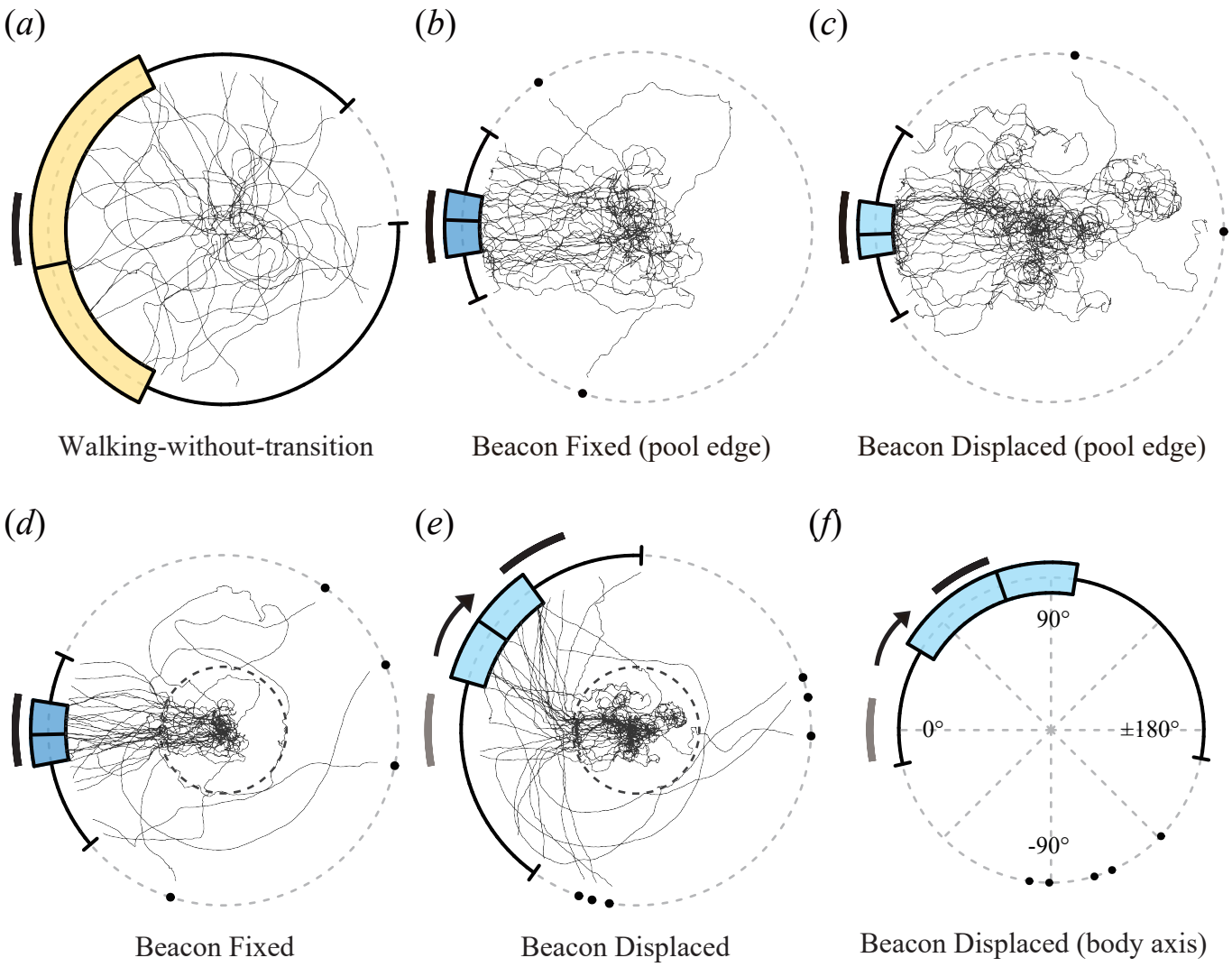

Figure 3. (a–e) Trajectories and circular box-and-whisker plots of final bearings are shown under the following conditions: Walking-without-transition condition, until ants reached the arena edge (a, *N*=33); Beacon Fixed condition, until ants reached the pool edge (b, *N* = 33); Beacon Displaced condition, until ants reached the pool edge (c, *N* = 33); Beacon Fixed condition, until ants reached the arena edge (d, *N* = 33); and Beacon Displaced condition, until ants reached the arena edge (e, *N* = 33), following the format of figure 2. For the condition where the beacon was displaced by 60° (e), an arrow indicates the direction of the beacon shift. The initial beacon range is shown in grey, the final beacon range after displacement in black. (f) Circular box-and-whisker plots illustrate the distribution of the body axis direction (from abdomen to head) at the arena edge under Beacon Displaced condition, as described in (e).

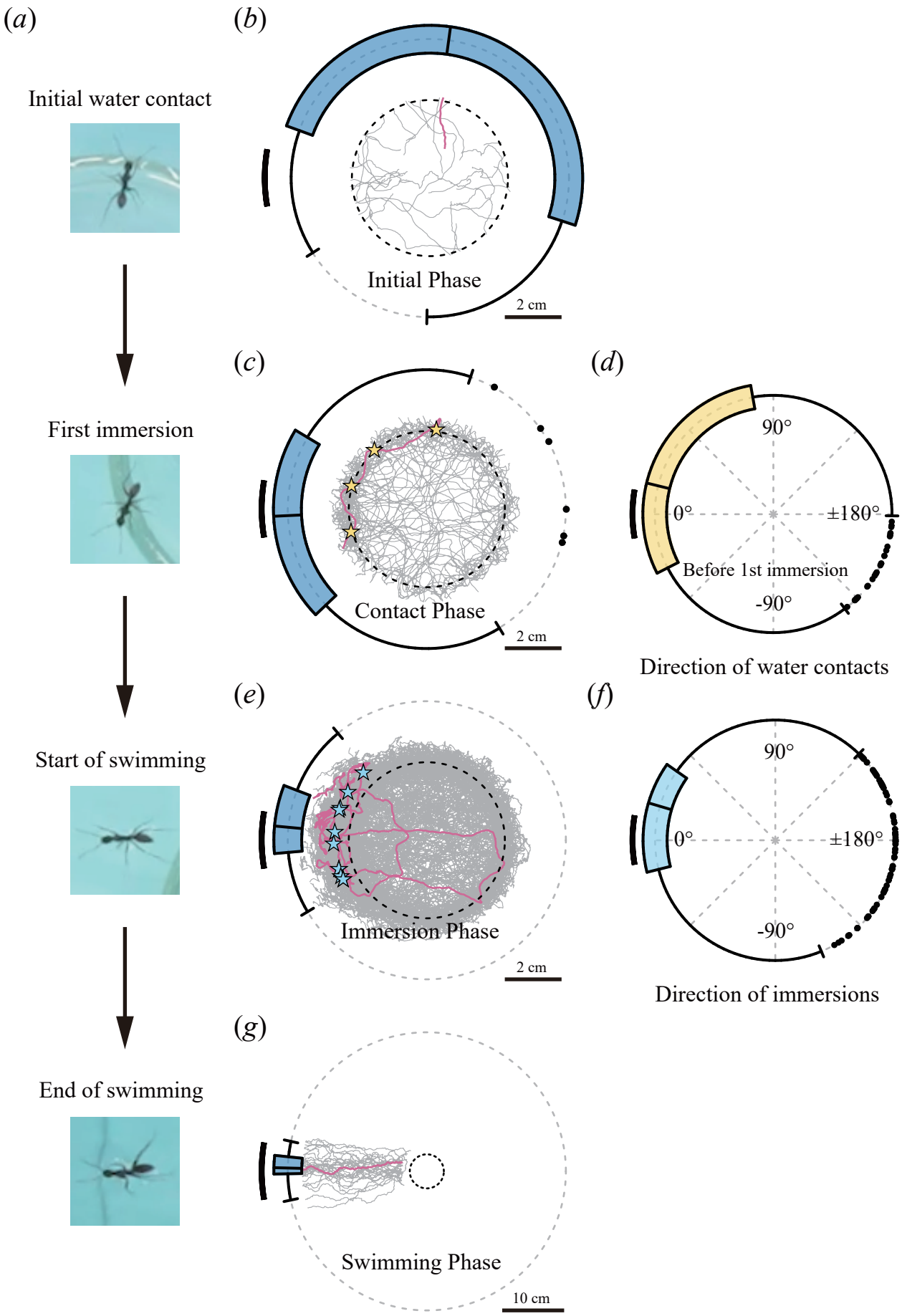
(a)
Initial water contact
First immersion
Start of swimming
End of swimming
(b)
Initial Phase
2 cm
(c)
Contact Phase
2 cm
(d)
90°
0°
±180°
Before 1st immersion
-90°
Direction of water contacts
(e)
Immersion Phase
2 cm
(f)
90°
0°
±180°
-90°
Direction of immersions
(g)
Swimming Phase
10 cm

Figure 4. (a) Representative frame images capturing key moments: Initial water contact, First immersion, Start of swimming, and End of swimming. (b, c) Trajectories during the Initial Phase (b) and Contact Phase (c) are displayed along with circular box-and-whisker plots indicating the directions of initial water contacts for all ants (N = 33), following the format of Figure 2. A representative trajectory is highlighted in magenta, and the water contact positions during the Contact Phase are marked with light yellow stars. Island edges are shown as black dotted circles, and the trajectory scales are located at the bottom right of each plot. (d) A circular box-and-whisker plot illustrates the directional distribution of all water contacts made by ants before the first immersion, formatted as in Figure 3. (e) Trajectories during the Immersion Phase are presented with circular box-and-whisker plots of initial water contacts, consistent with (b). A representative trajectory (magenta) is included, and positions of all immersions are indicated by light blue stars. (f) A circular box-and-whisker plot shows the distribution of water immersion directions before the ants started swimming, as in (d). (g) Trajectories during the Swimming Phase are depicted with circular box-and-whisker plots showing the final swimming bearings. The format is consistent with (b), and the representative trajectory (magenta) is included.