Born to be asocial: newly hatched tortoises avoid unfamiliar individuals

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Recognition of familiar individuals is important for modulating social interactions, but it is not clear to what extent this capacity depends on experience gained through repeated interactions with different animals. In wild tortoises, evidence of social interactions is limited to behaviours performed years after hatching, in the context of mating. To investigate the capacity to recognize familiar individuals at the onset of life in tortoises, we used hatchlings of two species (Testudo marginata, Testudo graeca) reared with a single conspecific as their unique social experience. When in a novel environment with the familiar conspecific, tortoises reached the distance expected after running random trajectories. In contrast, tortoises tested with an unfamiliar conspecific first explored the other tested individual, then actively kept a distance from it significantly larger than expected by chance. These results show evidence of spontaneous recognition of familiar individuals in a nonsocial species at the onset of life, and active avoidance of unfamiliar conspecifics. We suggest that this predisposition might be adaptive for young tortoises’ dispersal and that evolutionary pressures for social behaviour might be relevant for nonsocial species even at the onset of life.

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clear whether tortoises are capable of recognizing familiar individuals and if this capacity is present at the onset of life.

To investigate whether tortoises can spontaneously recognize familiar individuals, we used hatchlings of two tortoise species (Testudo marginata, Testudo graeca). Tortoises were hatched in individual compartments and raised with a single conspecific as a unique social experience, before being tested in a novel environment with a familiar tortoise (familiar condition) or an unfamiliar tortoise (stranger condition). We expected to observe different responses to familiar and unfamiliar individuals only if tortoises were capable of recognizing familiar individuals. By using a circumscribed arena, we were able to compare the average distance between tortoises with that expected with random trajectories and to evaluate whether tortoises stayed closer to or further from each other in the familiar and stranger condition.

METHODS

Subjects and Rearing Conditions

We observed 26 newly hatched tortoises: 14 T. graeca and 12 T. marginata individuals. Tortoises were about 1 month old (24–43 days of age, average 27 days) at the time of testing. Eggs laid on the ground by tortoises at the field station SperimentArea (Rovereto) were collected and incubated in darkness at 31 ± 2 °C. Tortoises hatched in individual compartments (3 × 4 cm) and were then moved to individual boxes (15 × 15 cm), with soil, leaves and straw covering the bottom, located outdoors (protected under a roof but exposed to daylight and partial shade). They were fed with green leaves ad libitum and gently hydrated with a spray watering can at least twice daily; this was sufficient to keep them well hydrated. The tortoises did not see any conspecific for about 10 days (2–20 days, average 10 days) before being paired with a tortoise of the same species. At this stage, each pair was housed in a square arena (20 × 20 cm and 12 cm high) with the bottom covered with soil, leaves and straw and maintained on the same regime as previously. Before the test, subjects did not see any other tortoise and did not interact with the experimenters.

Experimental Apparatus

As the experimental apparatus, we used a circular arena (25 cm in diameter and 10 cm high; Fig. 1a) with the bottom covered with wet sand (0.5 cm). A Windows LifeCam camera hung above the centre of the arena recorded the tortoises’ behaviour.

Procedure

We first familiarized tortoises with a conspecific by keeping a pair in the same enclosure for about 22 days (17–33 days, average 22.5 days). Tortoises were then tested in pairs of the same species (half of the pairs had the same mother, half had a different mother). Each tortoise was tested once or twice. The list of experimental pairs is shown in Table 1.

Before the beginning of the experimental session, we regulated the external temperature of the subjects by moving their box under a light, to make sure the temperature measured on the tops of the carapaces of the two tortoises differed by less than 2 °C. We measured the carapace temperature with an infrared thermometer. The average temperature of the pairs was 34.15 °C in the Familiar condition and 34.09 °C in the Stranger condition.

Immediately before the test, each individual was isolated for 5 min in an opaque box. Subsequently, the experimental subjects were placed in front of each other (a familiar or a stranger tortoise, according to the experimental condition), facing the centre of the arena, at diametrically opposed positions (the furthest possible distance within the arena). The Supplementary video shows an example of the experimental procedure.

The behaviour was recorded for 15 min from the moment when one of the two tortoises made the first step. We defined as the first step a movement of at least one leg that displaced the carapace. If neither tortoise moved within 10 min, the session was aborted and repeated the next day. To score the behaviour of tortoises, we extracted one frame every 20 s (3 frames/min) and used ImageJ (Rasband, 2017) to identify the location of the centre of the carapace and the tip of the head of each tortoise in all frames.

Figure 1. (a) Schematic representation of the experimental apparatus with a pair at the starting position. (b) Pairwise distance between heads (H) and carapaces (C). When $H < C$, tortoises face each other, when $H = C$ tortoises are parallel, facing the same direction, and when $H > C$ tortoises face away from each other.
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