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2 2014. Age-dependent social learning in a lizard. Biology Letters.

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4 **Extended Materials and Methods**

5 *Study animal and husbandry*

6 The Eastern Water Skink (*Eulamprus quoyii*) is a ground-dwelling lizard found throughout
7 much of southeastern Australia. Males of this species defend territories and adopt alternative
8 reproductive tactics and have been suggested to form dominance hierarchies [1, 2]. As such,
9 males tend to be visible to one another in the landscape but do not form groups. They are
10 capable of rapid spatial learning and do well in captivity [3]. We used *E. quoyii* from our
11 captive colony that was originally maintained in large semi-natural enclosures as part of a
12 mating experiment conducted in 2010 [2, 4]; young lizards were born in 2011. Heat cable
13 was placed under part of each lizard's enclosure to provide a thermal gradient. Enclosures
14 were lined with mulch substrate and each lizard was provided with a single refuge, a water
15 bowl, and thermoregulatory opportunities. Lizards were housed in these enclosures two days
16 prior to the beginning of the habituation stage and remained there for the duration of the
17 experiment (except for a few demonstrators; see below).

18

19 *Social demonstration experiments*

20 For logistical reasons we tested six 'old' and six 'young' lizards at a time (i.e. a single batch)
21 for a total of three batches. We randomly allocated lizards to treatments and balanced the
22 number of young and old lizards. In all experiments we conducted two trials per day, in the
23 morning (0900-1100h) and the afternoon (1300-1500h) with a minimum interval between
24 trials of 1.5 h (1.5-3 h). Trials were run every day for the duration of the experiment. Before
25 commencing experiments demonstrators were trained to remove the lid independently of
26 experimental lizards and we ensured that demonstrators were consistently removing lids

27 before commencing experiments. During this period, experimental lizards continued the
28 habituation stage I. If, during the experiment, the demonstrator lizard was not motivated to do
29 the task, we did not count the trial and we switched this demonstrator with a demonstrator
30 from a lizard in the control treatment (n = 3).

31

32 *Stage I. Habituation – feeding from a dish*

33 We placed a single petri dish on a block of wood and secured it in place using putty
34 (Bluetak®). Black, opaque electrical tape was wrapped around the sides of the dish so that
35 lizards could not use visual cues during the experiment. A single mealworm was placed in an
36 uncovered dish during each trial. Lizards had to eat from the open dish at least 6 times in a
37 row to move to the next stage. Mealworms were approximately 3 cm in length.

38

39 *Stage II. Instrumental task*

40 All trials were video recorded using a CCTV system (Avermedia with Sony 1/3” high
41 resolution color cameras) that enabled us to record the latency until the lid was displaced.
42 Lizards could either learn how to manipulate this lid from demonstrators in order to gain
43 access to the food reward, or learn by themselves. All trial videos were scored by a single
44 observer, however, it was not possible to score trials ‘blind’ because the camera captured
45 multiple lizards in the same frame. Given the lack of ambiguity of our variables (lizards
46 either displaced the lid or not) and because all trials were conducted without observer
47 interference, we doubt this confounded the results in any way.

48

49 *Stage III. Training – experimental lizards trained to manipulate lid*

50 Not all lizards learnt to displace the lid in the instrumental task. Therefore, prior to the
51 association task, lizards were trained to open the lid covering the dish in the instrumental

52 task. We used the same habituation procedures demonstrators experienced, except that we
53 switched to a two-dish habituation procedure, where one of the two dishes was three-quarters
54 covered and the second dish was fully covered. This expedited training of the instrumental
55 task, particularly for those lizards that were unsuccessful. One mealworm was placed in each
56 of the two dishes. A lizard was considered to have learnt how to shift the lid when it ate from
57 both dishes 5/6 times.

58

59 *Stage IV. Association task*

60 The association task required that lizards ($n = 36$) learnt to associate the food reward with a
61 dish covered by a blue lid. We randomized and counter-balanced the position of the blue dish
62 (right or left placement) to account for possible lateralization biases and then kept it in the
63 same position for every trial. We were therefore not able to test whether lizards used spatial
64 or colour cues to learn the task. We did this deliberately to expedite learning since our goal
65 was not to understand what cues lizards were using to learn tasks, but to determine whether
66 lizards were capable of social learning. To ensure that demonstrators were presenting
67 unambiguous information to experimental lizards in the social treatment we firmly attached
68 the white lid to prevent removal.

69 To test whether our learning criteria correctly categorized learners, we ran a subset of
70 lizards ($n = 21$) for five or more trials beyond their learning trial. We then tallied their overall
71 learning score to see whether it was significant according to a binomial choice test. In total
72 20/21 (95%) lizards with five or more trials beyond their learning trial (11-32 trials in total
73 per lizard; mean = 19.81 ± 1.40) retained a significant tally of choosing the blue lid in the
74 first try, strongly suggesting that our learning criterion was sufficient.

75

76

77 *Statistical analysis*

78 Data used for all analyses are located in the Dryad Digital Repository,
79 <http://datadryad.org/resource/doi:10.5061/dryad.6rj28> [5]. We analyzed the probability of
80 learning the instrumental task using generalized linear models (GLMs) with a binomial error
81 distribution ('logit' link). We included treatment and age as two-level categorical variables
82 along with an interaction between age and treatment because we hypothesized that there may
83 be differences in the use of social information between young and old lizards. We included
84 'batch' in our models to control for batch effects. Latency to open the dish and choose the
85 blue dish was first log transformed prior to analysis to ensure normality of residuals and
86 modeled using a Gaussian error distribution (identity link) using a GLM. To supplement
87 these analyses we also ran generalized linear mixed models (GLMMs) using all available trial
88 data for each individual. We treated correct and incorrect choices ('1' or '0') as coming from
89 a binomial probability distribution (i.e. binomial error – logit link) and included treatment,
90 age and a treatment by age interaction in the model. We also estimated a fixed effect for trial
91 to account for the fact that the probability of making a correct choice should increase across
92 trials for individual lizards because lizards were not rewarded for opening the white dish. In
93 all models we controlled for batch effects and included lizard ID as a random effect to
94 account for non-independence in measurements from the same lizard. To better account for
95 the correlation between residuals across trials within lizards, given the difficulty in estimating
96 random slopes, we re-ran our model using generalized estimating equations (GEEs) and
97 included an AR1 correlation structure to test whether temporal correlation affected our
98 estimates. This gave similar results to our GLMMs and thus we present results from our
99 random intercept model. Latency to choose the correct dish was modeled using a GLMM
100 with a Gaussian error distribution. In all cases models were simplified using likelihood ratio
101 tests by dropping each term from the full model and computing the χ^2 test statistics (binomial

102 or Poisson models) or F- statistics (Gaussian and quasipoisson/binomial) and their
103 corresponding p-values are presented for each predictor variable dropped from the final
104 model. Interactions were dropped first and then models were refitted and each term excluded
105 one at a time from the main effects model. In Poisson and binomial GLMs and GLMMs we
106 tested whether inferences were affected by over-dispersion (variance estimate deviates from
107 the mean) by re-running our top-supported models using either a quasipoisson/binomial error
108 distributions (GLMs) or including an observation level random effect (GLMMs). All
109 analyses were run using ‘lme4’ in R [6].

110

111 *Assessing motivation*

112 We considered whether lizards from the different treatments might have differed in their
113 levels of motivation because of a range of factors including age, mass and body size. First,
114 animals were completely randomized to each of the groups. Age is strongly related to both
115 mass and body size, as is the case in most lizards, and thus these variables were not included
116 in the final models. Although younger animals were smaller and lighter than older males, we
117 are confident that this did not lead to differences in motivation between groups. In the
118 association task, lizards did not eat in 9/797 (1.13%) trials based on all the lizards (young and
119 old) in the study. Furthermore, these ‘no eat’ trials were spread out across the groups (young-
120 social: 2; old-social: 5; young-control: 1; old-control: 1) and were not counted towards the
121 learning tally or in the models. In the instrumental task, which was much more difficult to
122 learn, we are also confident that motivation was not a driver in whether lizards removed the
123 lid or not. In the 12 trials prior to the start of the instrumental task (habituation to eating from
124 the dish and the 6 trials viewing prior to start of the task) only 3/36 lizards did not eat during
125 all of their trials. These lizards (1 young and 2 old) did achieve the learning criterion and also
126 ate very consistently in the association task. This is not what we would predict if there were

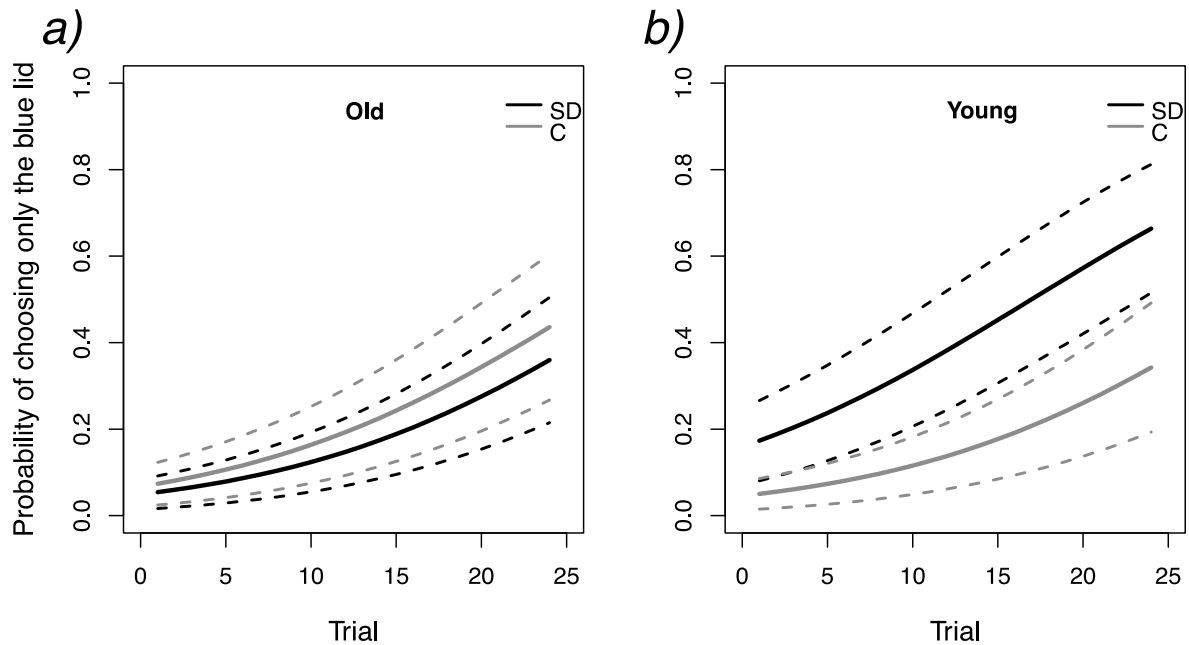
127 differences in motivation driving our effects, but rather we would expect to see older lizards
128 having far more trials where they did not eat, which was not the case. Based on these data we
129 are convinced that motivation was not an issue for our tasks.

130

131 **Results**

132 Given the age*treatment interaction, we have further analysed our data and tested for
133 significant differences between old lizards and young lizards in the control and social
134 demonstration experiments. As expected, old lizards in the social treatment were significantly
135 different (took more trials to learn) compared to young lizards in the social treatment ($t = -$
136 2.8 , $df = 11.2$, $p = 0.02$), while old lizards in the control treatment and young lizards in the
137 social treatment were not significantly ($t = -1.64$, $df = 13$, $p = 0.13$) different.

138



139

140 Fig S1 – Predicted probability of choosing only the blue lid (solid lines) and 95% confidence
141 intervals (dashed lines) for a) ‘Old’ lizards in the social demonstration (‘black’) and control
142 (‘gray’) treatments; b) ‘Young’ lizards in the social demonstration (‘black’) and control
143 (‘gray’) treatments.

145 **References**

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