

Supplementary material

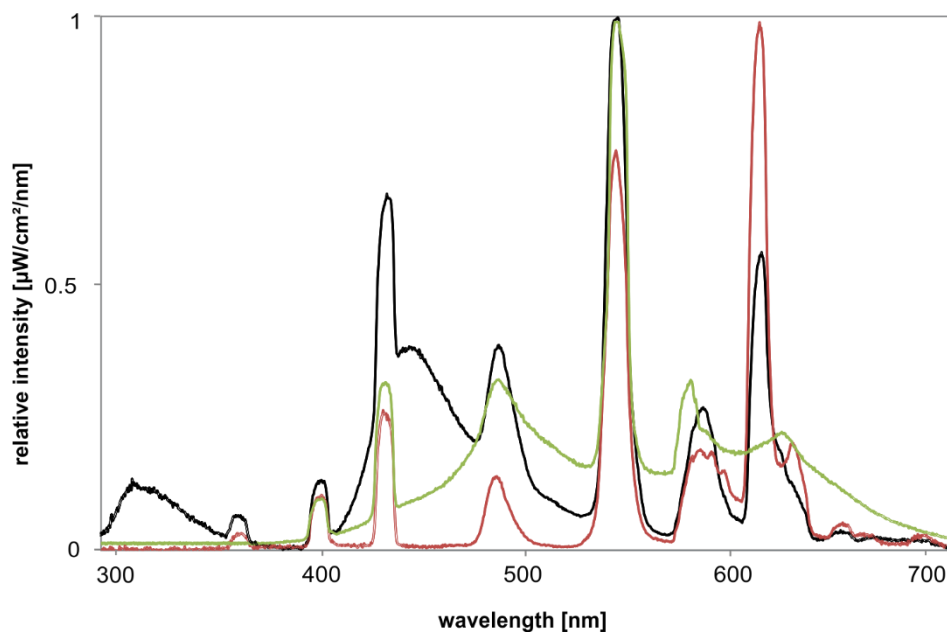


Fig. S1. Different environmental illumination conditions used in the experiments. Ants were kept under lights containing different quantities of UV. Red and green lines refer to illumination conditions where the ants were exposed during the colour preference, learning and memory experiments. Black line refers to illumination conditions containing high UV radiation (Repti Glo 2.0 full spectrum -with visible output- and Repti Glo 10.0 full spectrum -with UVB output- 15W, 45cm/18", Exo Terra, Holm, Germany); this combination was used for ants tested in the phototactic response and additional preference tests.

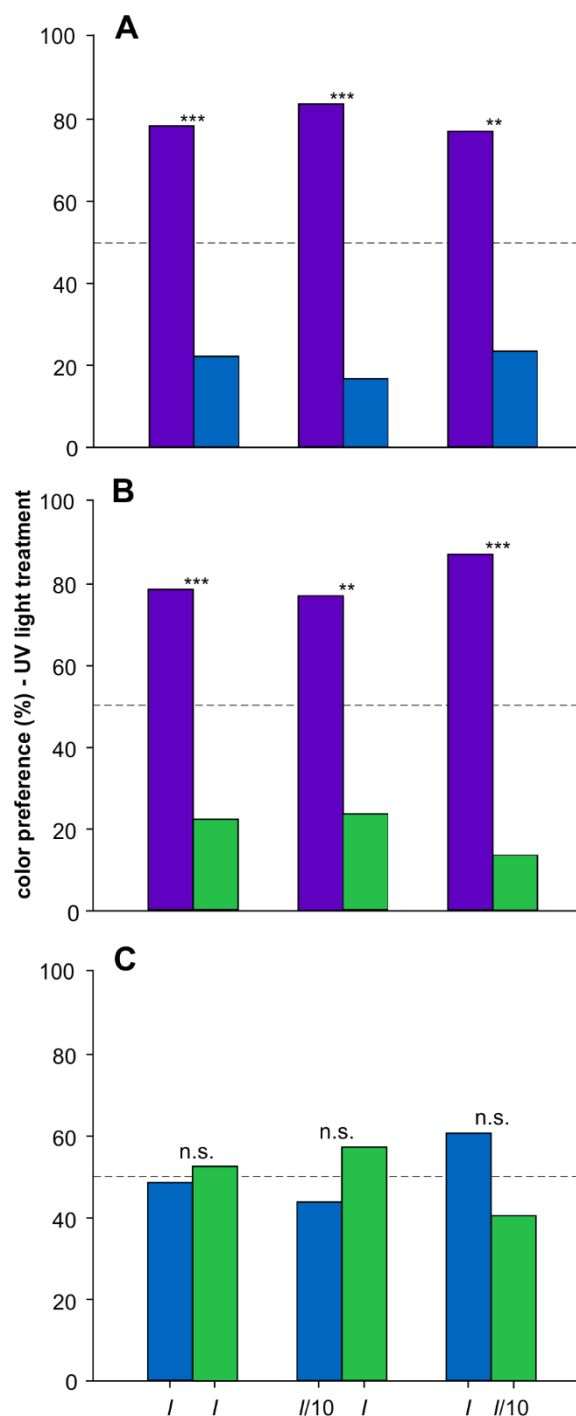


Fig. S2. Innate colour preference of colour-naïve foragers of *C. blandus* kept under different environmental lighting conditions containing high ultraviolet (UV) radiation. A-B. Colour-naïve foragers exhibit a strong innate preference for UV (365 nm) over blue (450 nm) and green (528 nm) light when presented at the same intensity [UV (*I*) vs. blue (*I*): $\chi^2 = 15.680$, $p < 0.001$; UV (*I*) vs. green (*I*): $\chi^2 = 15.680$, $p < 0.001$, $N = 50$ each] and when intensities differed by a factor of 10 [UV (*I/10*) vs. blue (*I*): $\chi^2 = 13.333$, $p < 0.001$; UV (*I*) vs. blue (*I/10*): $\chi^2 = 8.533$, $p = 0.003$; UV (*I/10*) vs. green (*I*): $\chi^2 = 8.533$, $p = 0.003$; UV (*I*) vs. green (*I/10*): $\chi^2 = 16.133$, $p < 0.001$, $N = 30$ each]. **C.** Ants did not show any significant preference for either blue or green light when presented at the same [blue vs. green: $\chi^2 = 0.08$, $p = 0.777$, $N = 50$] or at different intensities [blue (*I/10*) vs. green (*I*): $\chi^2 = 0.533$, $p = 0.465$; blue (*I* vs.

green ($I/10$): $\chi^2 = 1.200$, $p = 0.273$, $N = 30$ each]. Stimuli pairs emitted equal numbers of photons (UV vs. blue: $I = 1.3 \times 10^{13}$, UV vs. green: $I = 4.5 \times 10^{13}$, blue vs. green: $I = 8.2 \times 10^{12}$ photons/cm²*sec⁻¹) or differed by a factor of 10 ($I/10$). Significant differences are marked with asterisks. The dashed lines in the graphs indicate random choice. n.s., not significant; ** $p < 0.01$; *** $p < 0.001$. UV wavelength, purple bar; blue wavelength, blue bar; green wavelength, green bar.

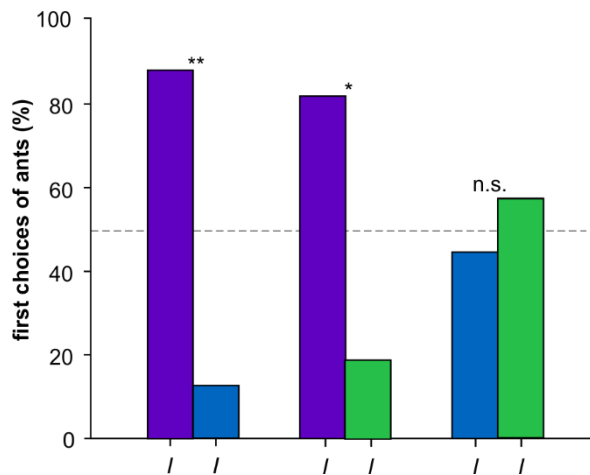


Fig. S3. First choice of colour-naive foragers of *C. blandus* during the first trial of learning experiments. Colour-naive foragers exhibit a strong innate preference for UV (365 nm) over blue (450 nm) or green (528 nm) light when presented at the same intensity, but show no preference when blue and green was presented [binomial test: UV (*I*) vs. blue (*I*): $p = 0.004$; UV (*I*) vs. green (*I*): $p = 0.021$; blue (*I*) vs. green (*I*): $p = 0.804$]. The dashed line in the graph indicates random choice. Significant differences are marked with asterisks. $N = 16$ individuals were analyzed for each stimulus pair. n.s., not significant; * $p < 0.05$; ** $p < 0.01$. UV wavelength, purple bar; blue wavelength, blue bar; green wavelength, green bar.

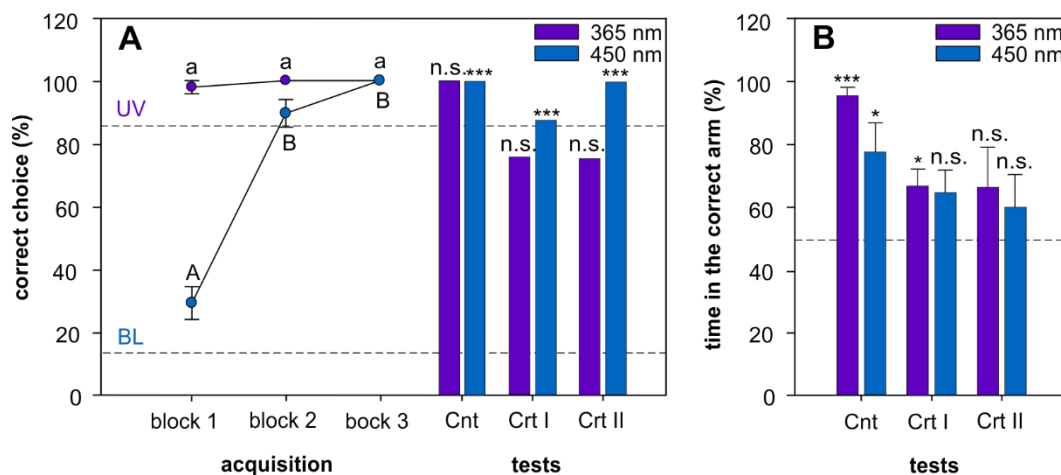


Fig. S4. Visual learning and discrimination performance of *C. blandus* foragers in a differential learning task when the intensity of the rewarded wavelength was decreased by a factor of 100 or 1000 (third learning group). **A.** shows the learning performance (learning curve with circles) and choice frequencies (bars). **B.** shows the time spent in the correct arm for the rewarding wavelength. Stimulus pairs emitted equal numbers of photons (training and control tests) or rewarded (CS+) wavelength was lowered by a factor of 100 (critical test I) or 1000 (critical test II). **A.** The proportion of the correct choices was high from the very beginning of the training when the UV light was presented as the rewarded wavelength (choice rate for UV within first choices: 100 %). Workers, however, could learn to discriminate blue from UV during 18 consecutive trials (12 training trials until the control test and 6 refreshment trials between the control and critical tests) [blue (CS+) vs. UV (CS-): $F_{2,14} = 79.308$, $p < 0.001$]. During the control and critical tests, ants could discriminate between rewarded (CS+) and unrewarded (CS-) conditioned stimulus, independent of which wavelength was rewarded or unrewarded. They chose the correct arm more often than expected compared to their innate preference (14% for blue) when blue was presented as CS+ [blue (CS+) vs. UV (CS-): Cnt: $p < 0.001$; Crt I: $p < 0.001$; Crt II: $p < 0.001$]. However, their UV preference (86 %) did not change when UV was presented as CS+ [UV (CS+) vs. blue (CS-): Cnt: $p = 0.299$; Crt I: $p = 0.311$; Crt II: $p = 0.311$]. **B.** Ants spent significantly more time in the arm with the rewarded colour in control and critical test I (but not critical test II) when UV was presented as the rewarded wavelength [UV (CS+) vs. blue (CS-): Cnt: $t_7 = 9.862$, $p < 0.001$; Crt I: $t_7 = 2.761$, $p = 0.028$, Crt II: $t_7 = 0.454$, $p = 0.664$, Fig. S4B] and only in control test when the blue was the rewarded wavelength [blue (CS+) vs. UV (CS-): Cnt: $t_7 = 2.808$, $p = 0.026$; Crt I: $t_7 = 1.900$, $p = 0.099$; Crt II: $t_7 = 1.023$, $p = 0.340$]. The dashed lines in the left-hand panel indicate the innate preference levels for the rewarded wavelengths determined in the preference tests (see Fig. 3). The dashed line in the graph in the right-hand panel indicates random choice. Significant differences (from the innate preference in A and the %50 level in B) are marked with asterisks. $N = 8$ individuals were trained and tested for each experimental group. BL, blue; Cnt, control test; Crt, critical test; n.s., not significant; UV, ultraviolet; * $p < 0.05$; *** $p < 0.001$. Error bars show standard errors. Different letters indicate significant differences between blocks.

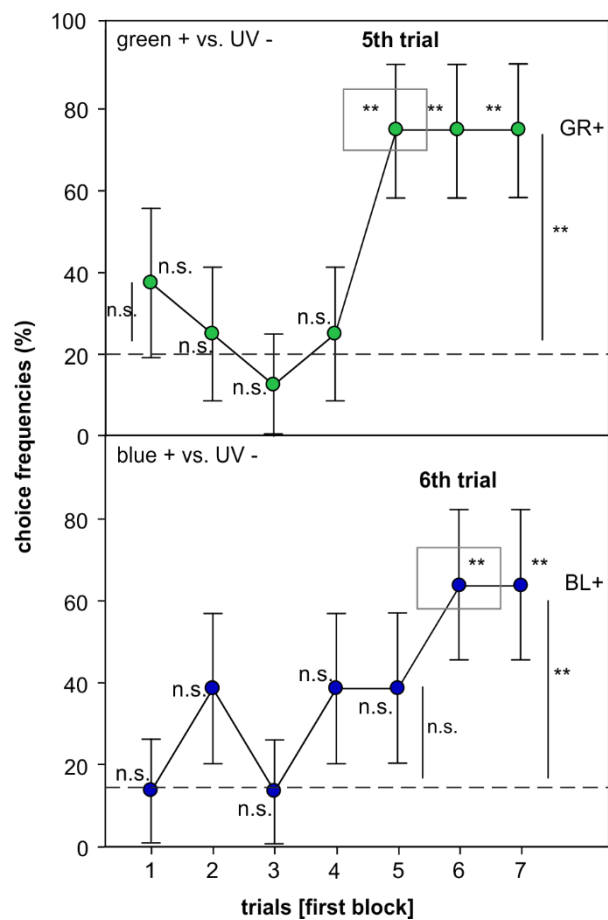


Fig. S5. Choice frequencies of the foragers during the first block of the learning experiments. Foragers learned the green vs. UV stimuli (upper part) faster (in 5th trial, binomial test: $p = 0.001$) than the blue vs. UV task (lower part, 6th trial, binomial test: $p = 0.002$). The dashed lines indicate the innate preference levels for the rewarded wavelengths determined in the preference tests (see Fig. 3). Error bars show standard errors.

Table S1. Stimulus intensity pairs used during the experiments

Phototactic response	UV	BL	GR
Higher intensity (color vs. dark)	1,32E+13	1,35E+13	1,30E+13
Lower intensity (color vs. dark)	2,16E+12	2,05E+12	1,29E+12
Color Preference	UV	BL	GR
Same intensity	1,32E+13	1,35E+13	
	4,50E+13		4,49E+13
		8,24E+12	8,24E+12
Lower intensity	2,16E+12	1,35E+13	
	1,32E+13	2,05E+12	
	1,21E+12		4,49E+13
	4,50E+13		1,29E+12
		7,03E+11	8,24E+12
		8,24E+12	8,03E+11
Colour learning and discrimination (First learning group)	UV	BL	GR
Training UV (CS+) vs. blue (CS-)	1,32E+13	1,35E+13	
Control	1,32E+13	1,35E+13	
Critical 1	2,16E+12	1,35E+13	
Critical 2	2,16E+12	2,05E+12	
Critical 3	2,16E+12	1,42E+14	
Training blue (CS+) vs. UV (CS-)	1,32E+13	1,35E+13	
Control	1,32E+13	1,35E+13	
Critical 1	1,32E+13	2,05E+12	
Critical 2	2,16E+12	2,05E+12	
Critical 3	1,42E+14	2,05E+12	
Training UV (CS+) vs. green (CS-)	4,50E+13		4,49E+13
Control	4,50E+13		4,49E+13
Critical 1	1,21E+12		4,49E+13
Critical 2	1,21E+12		1,29E+12
Critical 3	1,21E+12		8,08E+13
Training green (CS+) vs. UV (CS-)	4,50E+13		4,49E+13
Control	4,50E+13		4,49E+13
Critical 1	4,50E+13		1,29E+12
Critical 2	1,21E+12		1,29E+12
Critical 3	8,03E+13		1,29E+12
Training blue (CS+) vs. green (CS-)		8,24E+12	8,24E+12
Control		8,24E+12	8,24E+12
Critical 1		7,03E+11	8,24E+12
Critical 2		7,03E+11	8,03E+11
Critical 3		7,03E+11	5,54E+13
Training Green (CS+) vs. blue (CS-)		8,24E+12	8,24E+12
Control		8,24E+12	8,24E+12
Critical 1		8,24E+12	8,03E+11

Critical 2		7,03E+11	8,03E+11
Critical 3		5,27E+13	8,03E+11
Colour learning and discrimination (Second learning group)	UV (CS-)	BL (CS+)	GR (novel)
Training	1,32E+13	1,35E+13	
Control	1,32E+13	1,35E+13	
Critical 1	1,32E+13	2,05E+12	
Critical 2		1,35E+13	1,30E+13
Critical 3		2,05E+12	1,30E+13
Critical 4	1,32E+13		1,30E+13
Colour learning and discrimination (Second learning group)	UV (CS-)	GR (CS+)	BL (novel)
Training	1,32E+13	1,30E+13	
Control	1,32E+13	1,30E+13	
Critical 1	1,32E+13	1,29E+12	
Critical 2		1,30E+13	1,35E+13
Critical 3		1,29E+12	1,35E+13
Critical 4	1,32E+13		1,35E+13
Colour learning and discrimination (Third learning group)	UV	BL	GR
Training UV (CS+) vs. blue (CS-)	1,32E+13	1,35E+13	
Control	1,32E+13	1,35E+13	
Critical 1	1,88E+11	1,35E+13	
Critical 2	5,45E+10	1,35E+13	
Training blue (CS+) vs. UV (CS-)	1,32E+13	1,35E+13	
Control	1,32E+13	1,35E+13	
Critical 1	1,32E+13	1,90E+11	
Critical 2	1,32E+13	7,44E+10	
Visual (colour) memory	UV	BL	GR
Training blue (CS+) vs. UV (CS-)	1,32E+13	1,35E+13	
Control	1,32E+13	1,35E+13	
Critical 1	1,32E+13	2,05E+12	
Training Green (CS+) vs. UV (CS-)	4,50E+13		4,49E+13
Control	4,50E+13		4,49E+13
Critical 1	4,50E+13		1,29E+12