Supplementary information for

'Ripple effects' of urban environmental characteristics on cognitive processes in Eurasian red squirrels.

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Note S1. Detailed results of the generalisation latency across successes

In the generalisation problem, the repeated innovators showed significant improvement across the generalisation latency ($\chi^2_1 = 13.08$, P < 0.001). Accordingly, we further examined the innovators' latency by conducting success-by-success comparisons. We found that compared with their generalisation latency on the first success (i.e., their first generalisation latency), the repeated innovators showed their first significant improvement on the 3rd success (1st vs. 3rd: $Z = -2.36$, P = 0.018) and they consistently solved the problem using low latency from the 5th success onward (1st vs. 5th: $Z = -2.66$, P = 0.008; 1st vs. 6th: $Z = -3.25$, P = 0.001; 1st vs. 7th: $Z = -2.38$, P = 0.017; 1st vs. 8th: $Z = -2.94$, P = 0.003; 1st vs. 9th: $Z = -2.76$, P = 0.006; 1st vs. 10th: $Z = -3.86$, P < 0.001).

Note S2. Individual identification and the number of squirrels in each site

To identify each individual as well as record the number of squirrels in each site, we used an established method (1) alongside mark-recapture and mark-resight method. We subjected each video footage to a frame-by-frame analysis using Adobe Premiere Pro CS6. These video footage were records of video cameras that were set 1 m away from the apparatus in each field site. When we saw a squirrel the first time, it was 'marked' using their individual characteristics. It was 'recaptured' when it reappeared in the subsequent videos. We recorded each squirrel's characteristics as detail as possible that included their facial marking (e.g. a white dot/patch on face), colouration (e.g. orange, burgundy, brown patch on forehead), colour of limbs (e.g. orange/dark brown paws or dots on a toe) alongside height (relative to the apparatus), tail and body shape (e.g. full fur tail, half tail).

The first identification required intensive observer training that lasted for two months. Each squirrel was assigned a name and an identification number. This process required back and forth watching different footage so that the individuals' full characteristics could be revealed from different angles. We reidentified the squirrels three to five months after the first identification during which the same coder re-conducted the frame-by-frame analyses of the unmarked individuals by randomly selecting 3 out of 6 sites (total 23 individuals). To examine agreement between the two measures, we ran an intra-rater reliability test using Cohen's Kappa (Kappa = 0.99).

For marked individuals, we cross-checked the identity of individuals (with ear-tagged and collar) in five (out of 11) study sites that had ongoing field survey and trapping records (Table S1). Mark-resight method was used to confirm our observation. This method required walking slowly on the pathway in a site, or standing still in bushes or among trees to observe the squirrels. A squirrel was 'marked' using its unique characteristics mentioned above. We noted 'resight' when we saw the same squirrel. The double methods used here allowed us to check the identity of individuals in each site as well as to include the squirrels that were on trees.

Note S3. Detailed measurements of each urban environmental characteristic.
Chow and colleagues (2) have identified four urban environmental characteristics that were important for squirrels. These included the number of humans in a site (direct human disturbance), number of buildings around a site (indirect human disturbance), green coverage, and squirrel population size.

Direct human disturbance. We recorded the number of humans in a site 4-5 times daily regardless of weather conditions. In each record, we noted the time, weather, and the number of humans in a site before we set up the experiment in a site, before or after re-bating the apparatus, and when the experiment ended for the day, and thus resulting in 4-5 data points per day. Each record was obtained either from in-site recording or using distance-based methods. In-site recordings included walking around the site and counting each human that walked past the experimenter. This allowed us to count the number of humans more accurately if a site had a large area of bushes. Distance-based records meant dividing a site into four roughly equal areas and counting the number of humans in each area at the centre of the site or at the outer edge of the site, which minimises double counting. As there were other behavioural experiments that continued after the squirrels participated in the novel problem, we continued to obtain data on the number of humans in each site daily. We carried out an average of 38 observation days in each site (ranging from 33-42 days). We divided the total number of humans across the daily 4-5 scans in a site and across all observation days by the number of observation days to obtain the mean number of humans in a site per day.

Indirect human disturbance. Indirect human disturbance was the the number of human-built structures (e.g. schools, houses, restaurants and stores) within and 50 m surrounding each site. This 50 m covered urban red squirrels' minimum routine movement (3) where they can encounter anthropogenic food as well as capture the greatest human-induced disturbance as in different pollutants (e.g. noise, traffic, household and other human activities).

Green coverage. Green coverage of each site was defined as the areas covered by trees (m^2); a major resource that provides safety and food for squirrels. By using satellite mode of Google Map, we used point-to-point method, adding points around an area's boundary on the map and calculate the areas that were covered by trees site size (m^2)

Squirrel population size. Squirrels population size of each site was obtained using mark-recapture and mark-resight methods (see Note S1) that allowed us to double check the number of squirrels that we saw in the video footage and in each site.

Note S4. Detailed experimental procedures.

The field experimental protocol followed Chow and colleagues (2). Between May 2018 and January 2019, we tested 38 urban red squirrels in 11 sites (> 800 m between sites to avoid pseudo-replication) at Obihiro city, Hokkaido, Japan (see table S1 for site information). These sites were located at different places of the city, and vary with environmental characteristics of interest (e.g., direct and indirect human disturbance, areas of green coverage, squirrel population size). In these sites, squirrels are predominantly fed on Korean pine (*Pinus koraiensis*) and Manchurian walnut (*Juglans mandshurica* var. *sachalinensis*) trees.

The 38 squirrels were innovators who had previously repeatedly solved a novel food-extraction problem (i.e., the original problem, Fig. 1B). All squirrels were identified by an established method (1) that required frame-by-frame analysis of their characteristics from video footage as well as mark-recaptured and mark-resight methods (see Note S1). In addition to this, in five sites, the squirrels could be identified by their ear tags and/or collars from our long-term behaviour and population monitoring (4, 5).

3-5 days before starting the main experiment, we used hazelnut kernels to attract the squirrels to a location that was far away from major roads as well as close to a tree; this aimed to minimise road kill or predation risk (6). Once squirrels visited the location regularly (indicated by direct observation and emptied hazelnut kernels), we then presented the food-extraction problems (one box at a time) to the squirrels daily, during their most active period (from dawn to noon) to minimise possible confounding variables such as season, weather or motivation on performance.

During the experiment, we set the apparatus at where the original problem was and checked (i.e., rebait) it 3-4 times per day (45 mins-1.5 hours between checks). This inter-trial interval allowed us to to minimise social interference (less than 1% video had more than 1 squirrel on the apparatus at the same time) as well as recruit subordinate individuals when dominant individuals were at rest, and thus increased participation rate. In each check, we randomised the facing direction of the nut containers and the apparatus. For the generalisation task, the presentation sequence of the coloured levers was also randomised. For the memory test, which levers had a nut was chosen at random.

The field experiment lasted around 3.5 weeks in each site. All the innovators received the generalisation task the next day after they had completed the original problem. Before we presented the original problem to the innovators again, we carried out other behavioural assays that did not involve any similar solutions for solving the generalisation problem or the memory test. 21 days after the generalisation test, we presented the original problem to the innovators only for one day (from dawn to noon during their most active time); note that this has resulted in fewer successes obtained by each innovator in the memory test than in the generalisation problem.

Table S1. Urban environmental characteristics on participation rate at site level (N = 11) for the generalisation problem (A) and the memory test (B). Consider which environmental characteristics lead squirrels to participate in a task at the population level (general) and at the innovator level (after they learned something well). Participate rate was calculated as the squirrels who had solved the original problem more than once (the innovators) in the original problem and went on to the generalisation problem and the memory task; the number of innovators participating in a task divided by the number of innovators participated in the original problem. Urban environmental characteristics include direct human disturbance (the mean human in a site per day), indirect human disturbance (the number of buildings 50 m around a site), green coverage (the area in m^2 that is covered by trees or bushes) and squirrel population size (the number of squirrels in a site). Bold value indicates P < 0.05

	Response variable	Environmental characteristics	Estimate	S.E	Z	P
A	Proportion of innovators participated in the Generalisation problem	Direct human disturbance	0.17	0.38	0.44	0.661
		Indirect human disturbance	0.48	0.36	1.33	0.183
		Green coverage	0.39	0.36	1.08	0.282
		Population size	-0.68	0.35	-1.96	**0.050**
B	Proportion of innovators participated in the memory test	Direct human disturbance	-1.32	0.46	-2.83	**0.005**
		Indirect human disturbance	-0.91	0.41	-2.23	**0.026**
		Green coverage	0.54	0.38	1.42	0.155
		Population size	-0.10	0.35	-0.27	0.786

Table S2. Path analyses results that include all direct and indirect effects between urban environmental characteristics and the generalisation latency across successes in early learning (1st-5th successes). Environmental characteristics include direct human disturbance (DH), indirect human disturbance (IH), green coverage (GC) and squirrel population size (PS).

Response variable	Environmental characteristics	Mediator 1	Mediator 2	Effect	Total effect
Generalisation latency across early learning (1st-5th successes)	Direct human disturbance (DH)	DH		0.220	0.14
		IH		-0.004	
		GC		-0.077	
		PS		-2E-06	
		IH	GC	-0.002	
		IH	PS	<-0.001	
		GC	IH	-0.001	
		GC	PS	0.001	
		PS	IH	-2E-07	
		PS	GC	3.1E-06	
	Indirect human disturbance (IH)	IH		0.040	<-0.01
		DH		-0.070	
		GC		0.016	
		PS		0.004	
		DH	GC	0.025	
		DH	PS	5.6E-07	
		GC	DH	-0.008	
		GC	PS	<-0.001	
		PS	DH	-0.004	
		PS	GC	-0.007	
	Green coverage (GC)	GC		0.320	0.17
		IH		0.005	
		DH		-0.150	
		PS		-0.006	
		IH	DH	-0.009	

		IH	PS	0.001	
		DH	IH	0.003	
		DH	PS	1.2E-06	
		PS	IH	-0.001	
		PS	DH	0.006	
	Squirrel population size (PS)	PS		0.060	-0.06
		IH		0.008	
		DH		-0.057	
		GC		-0.106	
		IH	DH	-0.014	
		IH	GC	0.003	
		DH	IH	0.001	
		DH	PS	4.6E-07	
		GC	IH	-0.002	
		GC	DH	0.049	

Table S3. Path analyses results that include all direct and indirect effects between urban environmental characteristics and the generalisation latency across successes in the late learning (6-10th successes). Urban environmental characteristics include direct human disturbance (DH), indirect human disturbance (IH), green coverage (GC) and squirrel population size (PS).

Response variable	Environmental characteristics	Mediator 1	Mediator 2	Effect	Total effect
Generalisation latency across late learning (6-10th successes)	Direct human disturbance (DH)	DH		-0.370	-0.32
		IH		0.033	
		GC		0.005	
		PS		-1E-06	
		IH	GC	<0.001	
		IH	PS	<-0.001	
		GC	IH	0.010	
		GC	PS	0.001	
		PS	IH	1.9E-06	
		PS	GC	-2E-07	
	Indirect human disturbance (IH)	IH		-0.330	-0.19
		DH		0.118	
		GC		-0.001	
		PS		0.004	
		DH	GC	-0.002	
		DH	PS	4.7E-07	
		GC	DH	0.013	
		GC	PS	<-0.001	
		PS	DH	0.007	
		PS	GC	<0.001	
	Green coverage (GC)	GC		-0.02	0.17
		IH		-0.043	
		DH		0.252	
		PS		-0.005	
		IH	DH	0.015	

		IH	PS	<0.001	
		DH	IH	-0.022	
		DH	PS	9.9E-07	
		PS	IH	0.007	
		PS	DH	-0.010	
	Squirrel population size (PS)	PS		0.05	0.03
		IH		-0.066	
		DH		0.096	
		GC		0.007	
		IH	DH	0.024	
		IH	GC	<-0.001	
		DH	IH	-0.009	
		DH	PS	-2E-07	
		GC	IH	0.014	
		GC	DH	-0.083	

Table S4. Path analyses results that include all direct and indirect effects between urban environmental characteristics and the first memory latency. Urban environmental characteristics include direct human disturbance (DH), indirect human disturbance (IH), green coverage (GC) and squirrel population size (PS).

Response variable	Environmental characteristics	Mediator 1	Mediator 2	Effect	Total effect
Generalisation latency across early learning (1st-5th successes)	Direct human disturbance (DH)	DH		-0.520	-0.45
		IH		0.038	
		GC		0.009	
		PS		0.011	
		IH	GC	0.002	
		IH	PS	-0.003	
		GC	IH	0.006	
		GC	PS	0.005	
		PS	IH	-0.002	
		PS	GC	0.001	
	Indirect human disturbance (IH)	IH		-0.250	-0.08
		DH		0.140	
		GC		-0.011	
		PS		0.017	
		DH	GC	-0.002	
		DH	PS	-0.003	
		GC	DH	0.039	
		GC	PS	-0.007	
		PS	DH	-0.008	
		PS	GC	0.001	
	Green coverage (GC)	GC		-0.050	0.09
		IH		-0.035	
		DH		0.187	
		PS		-0.031	
		IH	DH	0.020	
		IH	PS	0.002	

		DH	IH	-0.014	
		DH	PS	-0.004	
		PS	IH	0.005	
		PS	DH	0.014	
	Squirrel population size (PS)	PS		0.280	0.11
		IH		-0.048	
		DH		-0.123	
		GC		0.018	
		IH	DH	0.027	
		IH	GC	-0.002	
		DH	IH	0.009	
		DH	PS	0.002	
		GC	IH	0.013	
		GC	DH	-0.067	

Table S5. Path analyses results that include all direct and indirect effects between urban environmental characteristics and memory latency across successes. Urban environmental characteristics include direct human disturbance (DH), indirect human disturbance (IH), green coverage (GC) and squirrel population size (PS).

Response variable	Environmental characteristics	Mediator 1	Mediator 2	Effect	Total effect
Generalisation latency across early learning (1st-5th successes)	Direct human disturbance (DH)	DH		-0.430	-0.42
		IH		0.024	
		GC		-0.026	
		PS		0.020	
		IH	GC	-0.004	
		IH	PS	-0.004	
		GC	IH	0.003	
		GC	PS	0.005	
		PS	IH	-0.002	
		PS	GC	-0.003	
	Indirect human disturbance (IH)	IH		-0.160	0.02
		DH		0.129	
		GC		0.026	
		PS		0.026	
		DH	GC	0.008	
		DH	PS	-0.006	
		GC	DH	0.019	
		GC	PS	-0.005	
		PS	DH	-0.015	
		PS	GC	-0.004	
	Green coverage (GC)	GC		0.160	0.25
		IH		-0.021	
		DH		0.116	
		PS		-0.030	
		IH	DH	0.017	
		IH	PS	0.003	

		DH	IH	-0.007	
		DH	PS	-0.005	
		PS	IH	0.003	
		PS	DH	0.017	
	Squirrel population size (PS)	PS		0.330	0.06
		IH		-0.037	
		DH		-0.185	
		GC		-0.053	
		IH	DH	0.030	
		IH	GC	0.006	
		DH	IH	0.010	
		DH	PS	-0.011	
		GC	IH	0.007	
		GC	DH	-0.038	

Table S6. Detailed information for the 11 study sites. This table is taken from Chow and colleagues (2021). Information include location (site name), using satellite mode of Google Map for GPS coordination and site size (m^2), surface area covered by tree canopy divided by site size (proportion of green area), participation rate in the original problem (the number of squirrels participating in the study divided by the actual squirrel population size in each site using mark-recapture and mark-resight methods) of each study location, and the number (and the type) of potential non-human predators recorded upon spotting one.

Location	GPS coordination	Site size (m^2)	Green area (proportion)	Squirrel population size (Participation rate%)	Potential non-human predators (e.g., raptors, foxes, domestic cats and dogs)
Manabino park	42.87, 143.19	46,433.4	0.34	9 (88.9%)	3 (foxes, cats, raptors)
Riverside	42.88, 143.18	68,162.6	0.71	9 (88.9%)	3 (foxes, raptors, cats)
Azusa park	42.93, 143.17	20,957.5	0.33	4 (100%)	2 (cats)
Nishiobihiro park	42.91, 143.13	40,686.1	0.60	9 (100%)	3 (foxes, cats and dogs)
Tsuda park	42.92, 143.12	85,769.6	0.74	7 (100%)	3 (cats and dogs)
Fushikobetsu park	42.92, 143.13	49,572.16	0.80	8 (87.5%)	2 (cats and dogs)
Ishio Ryokuchi park	42.91, 143.15	24,262.6	1	9 (100%)	4 (cats)
Oyama Ryokuchi park	42.90, 143.17	51,183.6	0.85	9 (100%)	3 (cats)
Obihiro Forest (Baseball field)	42.88, 143.15	171,859.5	0.73	6 (83.3%)	2 (foxes, cats)
Obihiro University	42.87, 143.17	388,390.6	0.09	5 (100%)	2 (cats)
Ozora park	42.88, 143.15	51,012.2	0.56	4 (50%)	2 (cats and dogs)

Table S7. Pearson's correlations between environmental characteristics correlations that include direct human disturbance, indirect human disturbance, squirrel population size, and green coverage at site level for <u>the proportion of success of the generalisation problem</u> (N = 11). Direct human disturbance was the mean number of humans in a site per day (i.e., the total number of humans from the 4-5 times of checks per day and across all observation days divided by the number of observation days). Indirect human disturbance was the number of human-built structures including shops, stores, schools, industries or houses within and 50 m surrounding each site. Green coverage (m^2) was measured as the actual area (m^2) covered by trees in each site on Google satellite. Squirrel population density was measured as the number of individuals divided by the site area (m^2). Site size (m^2) was the actual area (m^2) of a site on Google satellite. Because population density was moderately correlated with other variables of interest, we reran the correlation with population size (the number of individuals residing in a site) as an alternative variable to be included in the model. Bolded values indicate moderate to high correlation ($r \geq 0.5$) between the two variables.

	Indirect human disturbance	Green coverage (m^2)	Squirrel population density	Squirrel population size	Site size
Direct human disturbance	-0.30	-0.34	-0.10	-0.12	**0.52**
Indirect human disturbance		-0.06	**0.57**	0.01	-0.31
Green coverage (m^2)			**-0.54**	-0.05	0.32
Squirrel population density				**0.56**	**-0.66**
Squirrel population size					-0.37

Table S8. Pearson's correlations between environmental characteristics that include direct human disturbance, indirect human disturbance, squirrel population size, and green coverage for the model <u>generalisation latency on the first success, and latency across successes</u>. Direct human disturbance was the mean number of humans in a site per day (i.e., the total number of humans from the 4-5 times of checks per day and across all observation days divided by the number of observation days). Indirect human disturbance was measured as the number of buildings (e.g., shops, household, schools etc) 50 m around each area. Squirrel population size was measured as the number of squirrels residing in a site. Green coverage (m^2) was measured as the areas that are covered by trees or bushes in a site. Bolded values indicate $r >$ 0.5

	Indirect human disturbance	Squirrel population size	Green coverage (m^2)	Population density
Direct human disturbance	-0.31	0.04	-0.35	0.03
Indirect human disturbance		0.33	0.07	**0.60**
Squirrel population size			-0.40	**0.71**
Green coverage (m^2)				**-0.62**

Table S9. Pearson's correlations between environmental characteristics correlations that include direct human disturbance, indirect human disturbance, squirrel population size, and green coverage at site level for <u>the proportion of success of the memory test</u> (N = 9). Direct human disturbance was the mean number of humans in a site per day (i.e., the total number of humans from the 4-5 times of checks per day and across all observation days divided by the number of observation days). Indirect human disturbance was the number of human-built structures including shops, stores, schools, industries or houses within and 50 m surrounding each site. Green coverage (m^2) was measured as the actual area (m^2) covered by trees in each site on Google satellite. Squirrel population density was measured as the number of individuals divided by the site area (m^2). Site size (m^2) was the actual area (m^2) of a site on Google satellite. Because population density was moderately correlated with other variables of interest, we reran the correlation with population size (the number of individuals residing in a site) as an alternative variable to be included in the model. Bolded values indicate moderate to high correlation ($r \geq 0.5$) between the two variables.

	Indirect human disturbance	Green coverage (m^2)	Squirrel population density	Squirrel population size	Site size
Direct human disturbance	-0.31	-0.38	0.29	0.32	-0.28
Indirect human disturbance		0.15	**0.55**	0.30	-0.02
Green coverage (m^2)			**-0.64**	-0.38	**0.97**
Squirrel population density				**0.67**	**-0.72**
Squirrel population size					-0.44

Table S10. Pearson's correlations between environmental characteristics that include direct human disturbance, indirect human disturbance, squirrel population size, and green coverage for the model <u>memory latency on the first success, and latency across successes</u>. Direct human disturbance was the mean number of humans in a site per day (i.e., the total number of humans from the 4-5 times of checks per day and across all observation days divided by the number of observation days). Indirect human disturbance was measured as the number of buildings (e.g., shops, household, schools etc) 50 m around each area. Squirrel population size was measured as the number of squirrels residing in a site. Green coverage (m^2) was measured as the areas that are covered by trees or bushes in a site. Bolded values indicate $r >$ 0.5

	Indirect human disturbance	Squirrel population size	Green coverage (m^2)	Population density
Direct human disturbance	-0.26	0.26	-0.35	0.36
Indirect human disturbance		0.27	0.16	0.47
Squirrel population size			-0.47	**0.65**
Green coverage (m^2)				**-0.71**

Legends for Videos S1 and S2.

S1. A squirrel, Mario, was solving the original problem (see [here](#)), which was also used for the memory test.

S2. The squirrel, Mario, was solving the generalisation problem (see [here](#)), a task that was similar but novel to the original problem. We changed the shape of the apparatus and the colour of the levers to maximise the differences between the original problem and the generalisation problem. This task was used to assess the innovators' ability to apply a learned solution of the original problem to solve the generalisation problem.

Legends for Datasets S1 to S5.

S1. Dataset about participation and success rate at site level (S1. Success_Participation_datasets)

S2. Dataset of the first and last innovation latency as well as the first generalisation latency (S2. Generalisation_Paired_latency)

S3. Dataset of generalisation latency across successes (S3. G_first and across_solving latency)

S4. Dataset of the first and last innovation latency as well as the first memory latency (S4. Memory_paired_latency)

S5. Dataset of memory latency across successes (S5. M_first and across_solving latency)

SI References

1. P. K. Y. Chow, P. W. W. Lurz, S. E. G. Lea, A battle of wits? Problem-solving abilities in invasive eastern grey squirrels and native Eurasian red squirrels. *Anim. Behav.* **137**, 11–20 (2018).

2. P. K. Y. Chow, K. Uchida, A. M. P. von Bayern, I. Koizumi, Characteristics of urban environments and novel problem-solving performance in Eurasian red squirrels. *Proc. Biol. Sci.* **288**, 20202832 (2021).

3. K. Fey, S. Hämäläinen, V. Selonen, Roads are no barrier for dispersing red squirrels in an urban environment. *Behav. Ecol.* **27**, 741–747 (2016).

4. K. Uchida, K. Suzuki, T. Shimamoto, H. Yanagawa, I. Koizumi, Seasonal variation of flight initiation distance in Eurasian red squirrels in urban versus rural habitat. *J. Zool.* **298**, 225–231 (2016).

5. K. Uchida, T. Shimamoto, H. Yanagawa, I. Koizumi, Comparison of multiple behavioral traits between urban and rural squirrels. *Urban Ecosyst.* (2020) https:/doi.org/10.1007/s11252-020-00950-2.

6. C. M. Shuttleworth, *et al.*, Assessing causes and significance of red squirrel (Sciurus vulgaris) mortality during regional population restoration: An applied conservation perspective. *Hystrix* **26** (2015).