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Original Article

Decreased vigilance or habituation to humans? Mechanisms on increased boldness in urban animals

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Increased boldness is one of the most prevalent behavioral modifications seen in urban animals and is thought to be a coping response to anthropogenic environmental alterations. Most previous studies have shown enhanced boldness manifested as changes in responses to humans approaching, such as reductions in flight initiation distance (FID). However, this includes two confounding factors related to "boldness," that is, reduction of vigilance and habituation to humans. Confounding these totally different processes could lead to our misunderstanding of urban adaptation and how to properly manage urban wildlife. Here, we propose a simple framework to separate the two processes using two flight distance measures toward different approaching threats. We considered that the distance at which targeted individuals noticed an approaching object (i.e., alert distance, AD) was related to vigilance, whereas FID represented risk assessment, which is related to habituation. We applied a predictive framework using AD and FID to Eurasian red squirrels' responses to multiple threats of different risk levels (i.e., humans, model predators, and novel objects). AD was shorter in urban individuals compared with rural ones but not different among the approaching objects. FID was shorter in urban individuals and also varied among the objects with the shortest FID toward humans, whereas rural individuals showed similar FID to the different objects. These results suggest that, although urban individuals showed reduced vigilance, they could still assess different risk levels. Our framework can easily be applied to many animals and could significantly improve our understanding of wild animals' adaptations to urban environments.

Key words: **animal personality, cognition, human–wildlife interactions, neophobia, supplementary feeding, urbanization.**

INTRODUCTION

The rapid increase in urbanization over recent centuries has led to dramatic alterations in natural habitats (Gaston 2010). In order to cope with such disturbances, some animals have modified their behaviors and life histories, such as foraging mode, home-range area, temporal activity patterns, migration tendencies, and personality traits (Shochat et al. 2006; Tuomainen and Candolin 2011; Lowry et al. 2013; Gaynor et al. 2018). Increased boldness is one of the most widely reported behavioral shifts in many urban animal species (Møller 2008, 2012; Tuomainen and Candolin 2011; Lowry et al. 2013; Díaz et al. 2013). Because large predators avoid anthropogenic environments (Bateman and Fleming 2012), the predation risk in urban habitats tends to decline (Møller 2012; but see Loss and Marra 2017). As a result, prey animals become less vigilant, which is an adaptive response to urbanization (Lowry et al. 2013; but see Valcarcel and Fernández-Juricic 2009).

Increased boldness in urban environments is most frequently assessed by flight initiation distance (FID), the distance at which individuals start to flee from an approaching object (Short and Petren 2008; Møller 2010; Atwell et al. 2012). FID has a well-developed theoretical basis related to optimal escape theory (Ydenberg and Dill 1986; Cooper and Frederick 2007;

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Uchida et al. 2016) and can easily be applied to many species. Researchers simply approach target animals and measure how near they can get before their target flees. However, while FID can partly reflect a reduction in vigilance, a confounding factor exists, that is, habituation to humans (sensu Blumstein 2016). Literature relating to animal behavior or animal personalities often uses the term "boldness" (e.g., Réale et al. 2007) but rarely defines the terminology. Here, we considered that boldness consisted of two components: boldness in relation to general threats, which is related to vigilance, and boldness in relation to specific threats, which is related to habituation or risk assessment of specific threats. Confounding these totally different components could significantly bias the interpretation of any results. For example, the reduction of FID in response to a human approaching does not necessarily mean that an urban animal has lost vigilance or its antipredator response and, hence, become susceptible to novel mesopredators in urban environments such as cats (Geffroy et al. 2015). Surprisingly, very few studies have attempted to measure FID in response to nonhuman objects to understand how urban animals can assess different risk levels (but see McCleery 2009; Rodriguez-Prieto et al. 2009).

Here, we propose a novel framework for discriminating between the effects of a reduction in vigilance and habituation to humans on the increased boldness of urban animals. This will be accomplished by using two common measures of flight distance to compare responses between wildlife in urban and rural (natural) habitats toward multiple threats with different levels of risk (Figure 1). To investigate the effects of habituation to humans and the reduction in vigilance, we chose to use a human, a model predator, and a novel object (control) as approaching objects. Alert distance (AD) is the distance at which a target animal notices an approaching object and, therefore, should reflect a basic level of vigilance (Fernández-Juricic et al. 2003; Cooper and Blumstein 2014). Animals cannot identify what an approaching object is or how risky it is until they identify the object (e.g., via visual, olfactory, or auditory cues). Therefore, AD should not differ no matter what the approaching object is, and AD will be reduced when animals have lost vigilance in urban environments (Figure 1). FID, on the other hand, is the point at which a target animal decides to escape depending on the approaching risk and, therefore, should reflect risk assessment and habituation (Stankowich and Blumstein 2005; Cooper and Blumstein 2014; Samia et al. 2015). The greatest FID should be in response to

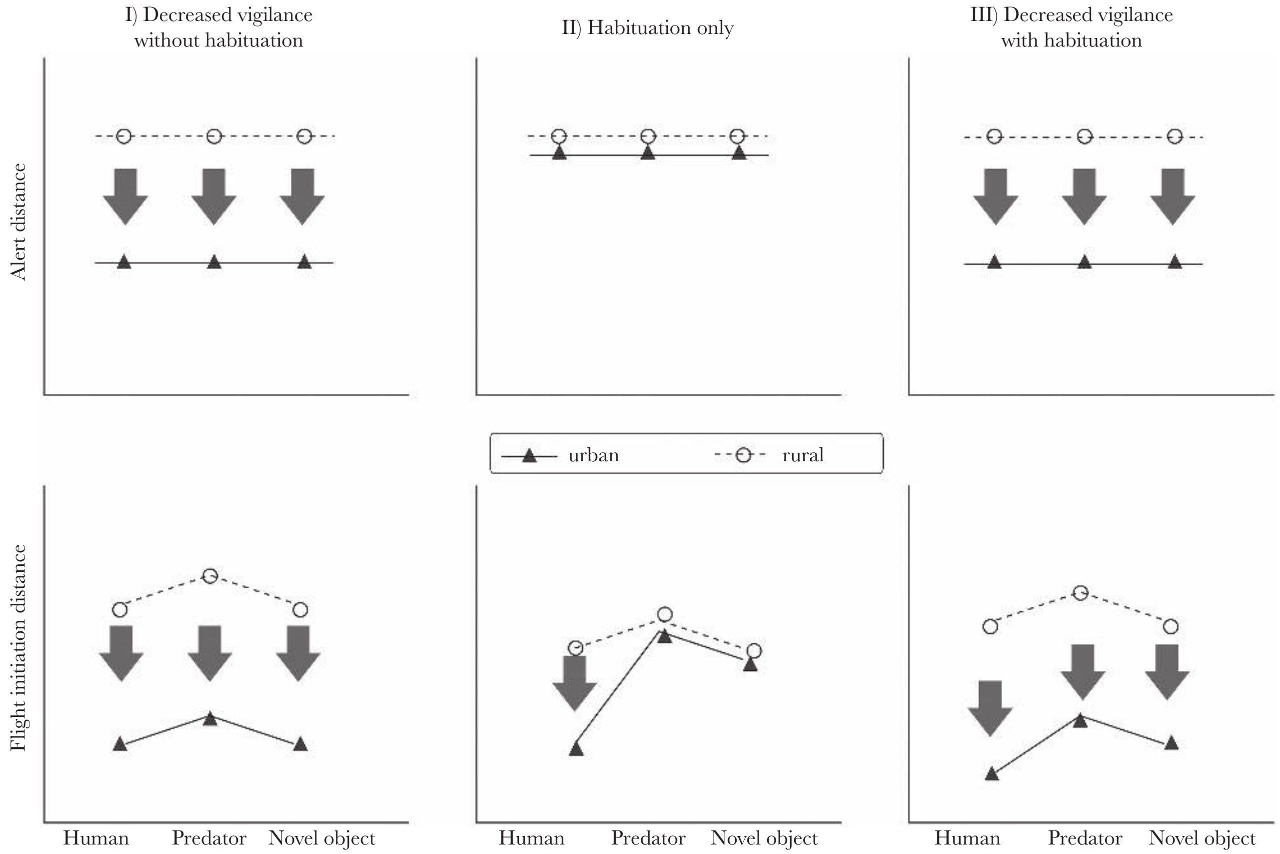

Figure 1

Predicted scenarios (I, II, and III) of increased boldness due to decreased vigilance and habituation to humans in urban animals. The *x* axis represents categories of potential threats; risk levels of each threat are predicted as Human < Novel object < Predator. The *y* axis represents AD and FID in response to the approach of three types of potential threats. The triangle and circle symbols illustrate the degree of flight distance of individuals in urban and nonurban (e.g., natural, rural) habitats, respectively. Scenario I represents the case where increased boldness is entirely due to decreased vigilance. AD and FID should be reduced in urban habitats, whereas FID in response to each threat should not differ. Scenario II represents the case where increased boldness is entirely due to habituation to humans. In this case, AD should not differ between urban and nonurbanized habitats, whereas FID should decrease in urban habitats in response to humans but not to predators and novel objects. Scenario III is the case where both decreased vigilance and habituation to humans contribute to increased boldness. In this case, both AD and FID should be modulated according to Scenarios I and II.

an approaching predator in natural conditions, and FID will be reduced specifically in response to humans in an urban environment if habituation has occurred (Figure 1). We proposed three alternative scenarios for increased boldness in urban animals (Figure 1). Scenario I: Increased boldness is entirely due to reduced vigilance. In this case, both AD and FID would be reduced in urban compared with rural habitats, whereas the order FID to each object will not change (e.g., longest for predator in both environments). Scenario II: Increased boldness is entirely due to habituation. In this case, AD will not differ between urban and rural habitats, whereas FID will be reduced only in response to a human approaching. Scenario III: Increased boldness is due to both processes. In this case, AD and FID will be reduced in urban habitats and the relative FID will also change.

We applied this framework to Eurasian red squirrels, *Sciurus vulgaris*, in central Hokkaido, Japan. Squirrels are a good model to understand how wild animals adapt to urban environments because many species have adjusted well to life in cities worldwide (Parker and Nilon 2008; Bateman and Fleming 2014; Uchida et al. 2016; Jokimäki et al. 2017). In addition, habitation to humans is highly likely because many squirrels in urban environments rely on artificial feeding by humans (Jokimäki et al. 2017).

MATERIALS AND METHODS

Study area and species

This study was carried out in the Tokachi region of central Hokkaido, Japan, between 5 and 27 October 2014. Obihiro is a large city (population about 160 000 people) located in the center of the Tokachi plain and is surrounded by rural agricultural lands. The mean temperature of Obihiro city during the study period was 9.0 °C and the daily maximum and minimum temperatures were 15.4 and 3.3 °C, respectively. We selected six city parks as our urban sites (1.19–50.47 ha) and four forests as our rural sites (1.78–25.0 ha). All sites were isolated from each other (at least 1 km away from the next habitat site). Individual squirrels rarely move among sites, especially in such a short period (less than 3 weeks). The urban and rural habitats differed in human population density, artificial structures (e.g., houses, buildings, and roads), and traffic levels. Urban habitats generally contained recreational playgrounds or pathways and were surrounded by dense residential areas and paved roads. Rural habitats, on the other hand, were surrounded by agricultural lands or near mountains. Although some rural habitats have man-made structures, we chose sites without such structures. Vegetation in urban parks, especially the underbrush, was cut lower than 50 cm by park managers. During our fieldwork period, vegetation at the rural sites had died back and, therefore, the visibility was similar in both urban and rural sites. Year-round artificial feeding by city residents was provided in all of the urban sites. In contrast, we have rarely encountered other people and never observed artificial feeding at the rural sites in the 4 years since we started our research on squirrels in these populations.

Red foxes (*Vulpes vulpes*) and raptors, such as the Eurasian hobby (*Falco subbuteo*), goshawk (*Accipiter gentilis*), and sparrowhawk (*Accipiter nisus*), are the main predators of red squirrels in Hokkaido in common with European countries (Selonen et al. 2010). These predators were likely much less common in urban habitats compared with rural habitats (author's personal observation). Domestic or stray cats, a potential novel predator in urban habitats (Jokimäki et al. 2017) were remarkably rare in our chosen urban habitats, whereas they were occasionally seen around farmer's houses in rural habitats. Domestic dogs were obliged to be kept on leashes, and we saw no stray dogs in the urban habitats.

Field survey

The field survey was carried out from sunrise (typically between 04:30 and 05:00 AM) to 10:00 AM, which is the time squirrels are most active (Ikeda et al. 2016). We randomized which sites were to be visited each day. We visited each site three times on average (range: 1–7). At each site, we targeted only individuals foraging on the ground. We identified individuals using their characteristics (e.g., body size, condition, and coat color) to avoid resampling. Only adult individuals were used for the experiment: juveniles (i.e., born in summer 2014) were identified with the external characteristics.

AD and FID were collected with a standard method (e.g., Bateman and Fleming 2011). To reduce the effects of observer bias on AD and FID, all data collection was conducted by the first author. Each squirrel was assigned to one of the following treatments: 1) human (the observer), 2) a stuffed red fox as a predator, and 3) a stuffed red fox covered with a black plastic bag as a novel object (or control). The stuffed fox and the novel object were fixed on a cart, which was attached to a long steel pole (6 m; Figure 2). When moving the objects on the cart with the pole, the observer was hidden behind an opaque board (95 × 65 cm), and it was noted that squirrels paid more attention to the objects than the board. The object was moved toward a squirrel at a constant speed of 1.0 m/s (i.e., human walking speed). Because the starting distance (SD) can potentially influence the flight distance (Rodriguez-Prieto et al. 2009), we tried to avoid the effects of SD on AD and FID by starting far enough for target animals not to identify an approaching object: significant correlations are expected only within a limited range and AD and FID reach plateaus in greater SD because animals cannot see the object (i.e., Zone III in Blumstein 2003; Cooper Jr and Blumstein 2014). Therefore, we recorded data only when the SD was 40 m or more, which was considered to be far enough for red squirrels (but see Results). When a target squirrel raised its head and looked toward the approaching object, the distance between the object and the squirrel was measured as AD (Uchida et al. 2017). We continued to approach the squirrel until it fled, at which point the distance was measured as FID. We marked the points of AD and FID by dropping tags while approaching a target squirrel and measured the distances once the trial had been completed. The distances were measured using a laser rangefinder (tru-Pulse 200, Laser technology Inc., Centennial, CO). Observations were not carried out on rainy, foggy, or windy days to avoid potential differences in squirrels' ability to detect threats. Data collection was also terminated when one of following occurred: 1) when targeted squirrels responded to alarm calls by conspecifics or avian species (e.g., Eurasian jays, *Garrulus glandarius*), 2) when pedestrians/bicycles approached targeted squirrels, and 3) when targeted squirrels approached the observer, possibly begging for food.

Data analyses

We performed two-way Anova to examine the effects of habitat (urban vs. rural), objects (human, predator, and novel object), and their interactions with AD and FID. Data were confirmed for normality (Kolmogorov–Smirnov test; for AD, $D = 0.13$, $P = 0.135$, and for FID, $D = 0.09$, $P = 0.242$) and homogeneity of variance (Levene's test for homogeneity of variance for AD and habitat, $F = 1.294$, $P = 0.280$; AD and object, $F = 4.031$, $P = 0.048$; FID

Figure 2
Image of the experiment. A predator model was fixed on a cart, which was attached to a long steel pole (6 m).

and habitat, $F = 1.345$, $P = 0.264$; FID and object, $F = 1.617$, $P = 0.206$). If the Anova result was significant ($P < 0.05$), we performed a post hoc test using Tukey's multiple comparison. All statistical analyses were carried out using R software, Version 3.0.1 (R Development Core Team 2015).

RESULTS

We recorded 81 AD (urban habitat: human $n = 17$, predator $n = 18$, novel object $n = 11$; rural habitat: human $n = 12$, predator $n = 14$, novel object $n = 9$) and 137 FID (urban habitat: human $n = 24$, predator $n = 29$, novel object $n = 25$; rural habitat: human $n = 25$, predator $n = 19$, novel object $n = 15$). Sample size was smaller in AD compared with FID mainly because it was sometimes difficult to identify when squirrels noticed (alerted) the approaching objects, especially when they were far away (Uchida et al. 2017). In some cases, highly vigilant squirrels might have already noticed the objects when we started data collection (>40 m apart). If this is true, we would have missed the AD data for such vigilant squirrels. To examine the possibility, we compared FID between the individuals whose AD were collected and those not collected (FID and AD were significantly correlated, Peason's correlation, $r = 0.663$, $P < 0.001$, $n = 81$). As a result, FID was significantly longer for individuals whose AD were not collected (linear regression model, AD collected or not: $P = 0.015$, Habitat, $P < 0.001$, AD × Habitat interaction, $P = 0.356$). Therefore, our AD was somewhat underestimated. Importantly, however, our main purpose is comparing between urban and rural habitats, as well as different approaching objects. Therefore, this bias should not change the conclusion of the results. The proportion of individuals whose AD were not collected was not different between urban and rural habitats (41% for urban and 39% for rural habitats, Fisher's Exact test, $P = 0.862$).

AD was significantly shorter in urban habitats compared with rural habitats (mean ± standard error [SE]: 19.9 ± 1.0 m for urban habitats, 27.6 ± 1.6 m for rural habitats), whereas no significant difference was observed among different objects (two-way Anova; Figure 3; Table 1). For FID, not only the habitat but also object and interaction effects were detected (Figure 3; Table 1). The mean FID of urban squirrels was just half that of their rural counterparts (mean ± SE: 9.6 ± 0.8 m for urban habitats, 19.1 ± 1.1 m for rural habitats). Tukey's multiple comparison test showed that the FID of urban squirrels in response to humans and predators was significantly reduced compared with rural squirrels (Figure 3; Table 2), whereas FIDs in response to the novel object were not significantly different. In addition, FIDs in response to humans were significantly shorter than for the model fox and novel object in urban squirrels but no significant difference was observed among the objects in rural habitats (Figure 3; Table 2). In short, the response to humans was the most notably modulated in urban habitats (mean ± SE: 4.5 ± 0.7 m for urban habitats, 18.5 ± 1.7 m for rural habitats) and FID was also reduced in response to the model predator (mean ± SE: 11.1 ± 1.0 m for urban habitats, 21.0 ± 1.8 m for rural habitats). Overall, the results were most consistent with Scenario III (Figures 1 and 3), although some deviations from the predictions were observed (i.e., reduction of FID in response to the predator model and no reduction of FID in response to the novel object).

DISCUSSION

Although it has been widely reported that urbanization increases the boldness of a range of animals (e.g., Møller 2008; Evans et al.2010; Samia et al. 2017), few studies have clarified the underlying processes. Here, we proposed a simple framework to separate two effects—a reduction in vigilance and habituation to humans—on increased boldness in wild animals by comparing AD and FID in response to multiple objects in both urban and rural habitats. We applied this framework to Eurasian red squirrels and found that both a reduction in vigilance and habituation contributed to their increased boldness in urban habitats. Because AD and FID are criteria that have been widely used in a variety of animals (Stankowich and Blumstein 2005), this novel framework could contribute to an

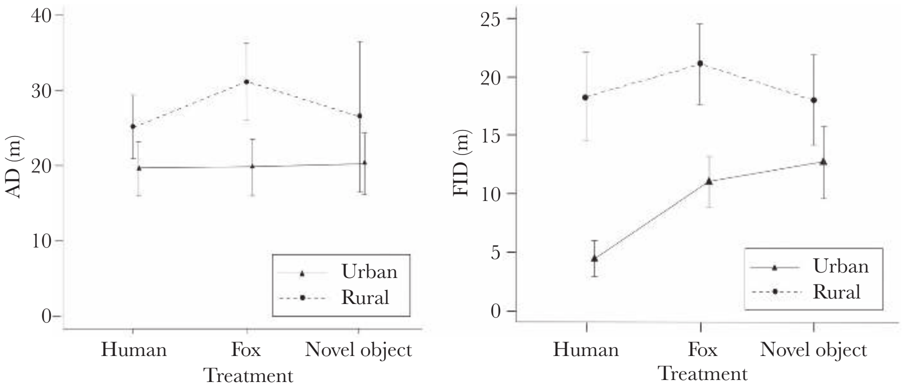

Figure 3

The result of AD and FID of urban and rural red squirrels in response to different objects. Means and standard deviations are shown. The sample size for AD of urban squirrels was 46 (human = 17, model fox = 18, and novel object = 11) and rural squirrels was 35 (human = 12, model fox = 14, and novel object = 9). The sample size for FID of urban squirrels was 78 (human = 24, model fox = 29, and novel object = 25) and rural squirrels was 59 (human = 25, model fox = 19, and novel object = 15).

Table 1

The results of Anovas for AD and FID between urban and rural squirrels in response to different approaching objects (human, model fox, and novel object)

		df	*F*	*P*
AD	Habitat (urban vs. rural)	1	17.574	<0.001
	Object (Human, Fox, and Novel)	2	1.216	0.302
	Interaction (Habitat × Object)	2	1.064	0.35
FID	Habitat (urban vs. rural)	1	73.19	<0.001
	Object (Human, Fox, and Novel)	2	3.606	0.03
	Interaction (Habitat × Object)	2	4.413	0.014

df, degrees of freedom.

Table 2

The results of Tukey's multiple comparisons of FID in squirrel responses to three objects in urban and rural habitats

	95% Confidence interval			
	Mean difference	Lower bound	Upper bound	*P*
Rural:Fox – Rural:Novel	3.01	−3.78	9.80	0.79
Rural:Human – Rural:Novel	0.50	−5.92	6.92	0.99
Rural:Human – Rural:Fox	−2.51	−8.49	3.47	0.83
Urban:Fox – Urban:Novle	−1.64	−7.00	3.72	0.95
Urban:Human – Urban:Novel	−8.25	−13.87	−2.63	<0.001
Urban:Human – Urban:Fox	−6.61	−12.03	−1.18	<0.01
Urban:Human – Rural:Human	−14.00	−19.62	−8.39	<0.001
Urba:Fox – Rural:Fox	−9.91	−15.71	−4.11	<0.001
Urban:Novel – Rural:Novel	−5.25	−11.67	1.17	0.18

increased understanding of urban adaptation and help to develop effective strategies for the management of urban animals.

Bold urban squirrels can assess different risk levels

Our conceptual model assumed reduced vigilance and habituation to be the main drivers of increased boldness among wild animals in urban habitats. AD, the distance at which an animal notices an approaching object, should reflect the animal's vigilance and should not depend on the approaching object. As expected, AD was not dependent on object type, although it was significantly reduced in squirrels living in urban compared with those in rural habitats, indicating a reduction in vigilance. Animals may notice a larger approaching object sooner than a smaller one. In our experiment, the predator model was much smaller (0.5 m in height) than a human, but the AD did not differ between the objects. Squirrels might first detect an approaching object via sound rather than vision. Regardless of a squirrel's perception systems, AD should be an appropriate indicator with which to assess vigilance. One drawback of measuring AD was the difficulty in identifying when animals noticed approaching objects, especially when they were far away. This is particularly important for highly vigilant species, such that they should already notice when we start data collection: thus, AD cannot be measured.

We also assumed that the distance at which an animal fled (FID) would depend on the type of approaching object and, therefore, reflect how familiar or risky the object is. The most remarkable result was the high tolerance of urban squirrels to humans approaching, which strongly suggested habituation. Humans are generally considered to be a top predator and act as stressors for many animals (e.g., "super predators"; Darimont et al. 2015). However, humans sometimes make affinity relationships with wild animals such as small birds and mammals; for example, humans feed animals and animals rely on such artificial feeding (Jones 2011; Jokimäki et al. 2017). In fact, during our field survey, squirrels often approached us and even climbed up our legs, probably begging for food (this never happened in rural habitats). Therefore, in order to better utilize resources provided by humans, tolerance to humans would be a big advantage in urban environments. FID in response to the predator model was significantly lower in urban squirrels compared with squirrels in rural habitats, whereas FID in response to the novel object did not differ between urban and rural squirrels. This was somewhat unexpected because if urban animals show increased boldness, they should also show a reduction in FID in response to novel objects. Or, if urban animals are still wary about something (e.g., novel objects), they should also be so for predators. There are two explanations for this disparity, which are not mutually exclusive. First, urban individuals may frequently encounter novel dangerous objects, such as cars and bicycles. Therefore, they might pay particular attention to unfamiliar objects. In fact, recent studies have shown neophobic tendencies in urban animals (e.g., Miranda et al. 2013; Audet et al. 2015; Federspiel et al. 2017). A second alternative explanation stems from visual category recognition: habituation to one stimulus may be transferred to other stimuli if both stimuli are classified as a similar type (Blumstein 2016; Geffroy et al. 2015). In our study sites, humans often walk their dogs in urban parks, and dogs usually do not approach squirrels either because of indifference or constrained by dog leashes. It is suggested that Eastern gray squirrels *Sciurus carolnensis* habituate also to dogs (Cooper et al. 2008). Because dogs and foxes are both medium-sized canids, habituation to domestic dogs might reduce the FID in response to the model fox (McCleery 2009).

Relatively few studies have compared flight responses of urban and rural animals toward multiple objects (McCleery 2009; Rodriguez-Prieto et al. 2009; Bateman and Fleming 2014; Weaver et al. 2018). By doing so, we clearly demonstrated that even a small rodent can properly assess different risk levels and that animals may possess higher cognitive abilities than was initially thought. Moreover, some recent studies have suggested that cognition in urban animals may be enhanced by rapidly changing urban environments (Griffin et al. 2017a). Because urban environments contain many novel stimuli introduced by human activities, animals may require high behavioral flexibility to better utilize urban resources (Sol et al. 2002, 2013; Griffin et al. 2017b). Although Eurasian red squirrels around Obihiro city showed decreased vigilance levels, they could still assess different risk levels. This flexibility may be one of the reasons why squirrels have been successful in colonizing urban habitats worldwide (Parker and Nilon 2008; Bateman and Fleming 2014; Uchida et al. 2016; Jokimäki et al. 2017).

Potential extension of the framework

Previous studies have shown increased boldness in urban animals and attributed the causes either to fewer predators or habituation to humans (Lowry et al. 2013). Because these mechanisms are totally different, it is necessary to separate them to better understand animals' adaptations to urban landscapes. Our approach enabled us to distinguish between alternative scenarios for increased boldness (Figure 1). This method also has the potential to quantitatively assess the relative effects of decreased vigilance and habitation to humans.

If we consider that FID consists of two components, decreased vigilance and habituation to humans (Figure 4), the former can be measured as a reduction in AD:

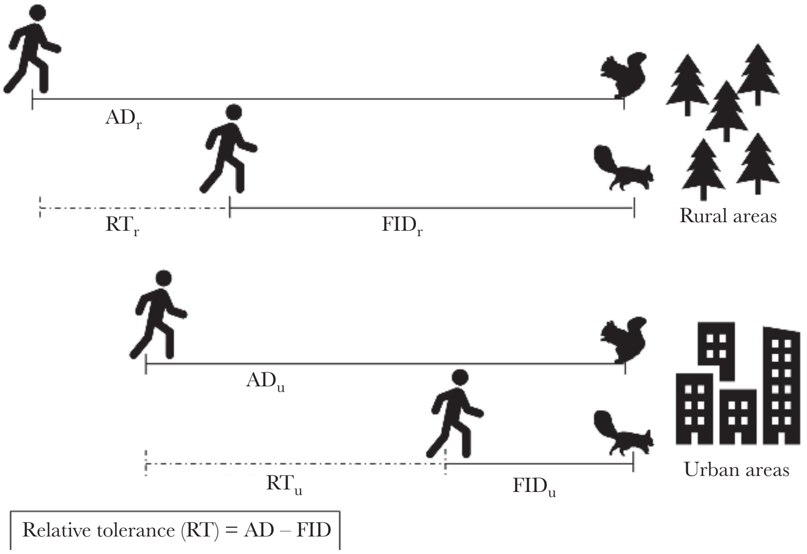

Figure 4

Illustration of the quantitative assessment of the relative strengths of decreased vigilance and habituation to humans using AD and FID between urban and rural habitats. The RT can be calculated as (AD − FID). The percentage of decreasing vigilance can be calculated as $(1 - AD_u/AD_r) \times 100$, and the percentage of habituation can be calculated as $(1 - [AD_u - FID_u]/[AD_r - FID_r]) \times 100$. More details are given in the main text.

Table 3

AD, RT, and FID in squirrels in urban and rural habitats in response to different approaching objects (i.e., human, model predator, and novel object). Data were based on individuals whose AD and FID were both collected. The relative strength reduction in vigilance and habituation to humans is calculated from the shift (by %) from rural to urban habitats for AD and RT, respectively. Because the distance animals recognize may be nonlinear, log-transformed values are also presented. See more details in the text

	Human			Model predator			Novel object		
	Rural	Urban	Shift by %	Rural	Urban	Shift by %	Rural	Urban	Shift by %
AD	25.1	19.7	21.4	31.2	19.9	36.3	26.5	20.3	23.6
RT	10.2	14.9	31.3	11.3	8.9	−26.5	9.5	10.2	7.3
FID	14.9	4.9	67.4	20.0	11.0	44.9	17.1	10.1	41.0
Log-transformed	Human			Model predator			Novel object		
	Rural	Urban	Shift by %	Rural	Urban	Shift by %	Rural	Urban	Shift by %
AD	1.4	1.3	7.5	1.5	1.3	13.1	1.4	1.3	8.2
RT	0.2	0.6	62.7	0.2	0.3	24.6	0.2	0.3	37.0
FID	1.2	0.7	41.5	1.3	1.0	19.9	1.2	1.0	18.6

$$\text{Decreased vigilance by } \% \ : (1 - \mathrm{AD}_u/\mathrm{AD}_r) \times 100$$

where the subscripts represent urban and rural, respectively. Habituation to humans, on the other hand, can be measured as the relative tolerance (RT or "buffer zone", Samia et al. 2017), that is, the distance the animal can bear after recognizing an approaching object (i.e., AD – FID, Figure 4):

$$\text{Habituation by } \% \ : (1 - [\mathrm{AD}_r - \mathrm{FID}_r]/[\mathrm{AD}_u - \mathrm{FID}_u]) \times 100$$

Note that the RT in urban habitat is in the denominator because urban individuals become more tolerate when habitation occurs.

When this calculation was applied to Eurasian red squirrels (based on the individuals where both AD and FID were measured), AD was reduced by 21.4% and RT was reduced by 31.3%, suggesting that habituation to humans may be 1.46 times more important compared with the reduction in vigilance (Table 3). These calculations are based on simple Euclidian distances but, in reality, for example, the same 5 m distance (assuming RT) would be different when the object is 10 or 30 m distant from the animals: actual tolerance should be higher when the initial distance is further away (e.g., Fleming and Bateman 2017). We could take such relative distance into account by log-transforming the data; that is by assigning a greater weight when the approaching object is closer. This suggests even stronger effects of habituation compared with reduced vigilance (i.e., 7.5% shift in AD and 62.7% shift in RT; Table 3).

Relative effects of habitation and reduced vigilance are not straightforward for the model predator and novel object. In Euclidian distances percentage shifts in AD were larger than those in RT, indicating stronger effects of reduced vigilance (Table 3). However, opposite patterns are generated if the distances were log-transformed. Log-transformation might impose too much weight in shorter distances. Appropriate calculations should be based on animal cognitive systems and further analytical development is certainly required. In addition, our calculation lacks highly vigilant squirrels in which we could not collect AD. Nevertheless, this method has a potential to quantitatively assess the relative roles of decreased vigilance and habituation on animal boldness. This simple measurement using the same unit (i.e., distance) also allows for a meta-analysis to include a wide range of urban animals.

The present approach could also contribute to the management of urban animals. It is often perceived that increased boldness in urban animals can increase their vulnerability to novel predators, such as domestic cats, and novel threats, such as vehicles (Geffroy et al. 2015). This should be true if increased boldness is largely due to decreased vigilance (Scenario I in Figure 1). However, when increased boldness reflects risk assessment (Scenario II in Figure 1), urban animals can probably avoid such negative effects. Measuring boldness by only using FID in response to humans approaching, the traditional method, cannot distinguish between these cases and may result in inappropriate management actions. In spite of the wide applicability and long history of flight distance studies, very little research has employed both FID and AD toward multiple approaching objects. Our framework is a promising approach to a better understanding of animals' adaptation to urban environments, as well as management of urban wildlife.

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Data were collected in a noninvasive manner (i.e., no squirrels were captured), and no part of the study violated ethical laws or the welfare of animals. All procedures were ethically approved by the Institutional Animal Care and Use Committee of National University Corporation Hokkaido University (license number 15–0121).

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Uchida et al. (2019).

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