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**The town squirrel and the country squirrel:
multiple behavioral comparisons in Eurasian red
squirrel between two environments**



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SUMMARY

- Human activities have been imposing novel challenges to animals. Especially, the urbanization, which leads dramatic environmental alterations, causes global scale biodiversity loss. While many species have disappeared because of urbanization, some species can survive or adjust to urban environments. Recent studies have revealed that such species modify behaviors to cope with anthropogenic disturbances, for example by increasing boldness and exploration. However, since past studies examined relatively small numbers of behavioral traits and were biased to avian species, we still do not fully understand how animals respond to human activity and urbanization. Behavioral responses to human activity should be species specific, and thus more empirical assessments are required.
- The aim of this thesis is to assess the multiple behavioral responses of Eurasian red squirrel *Sciurus vulgaris* towards urbanization. I compared flight distance and its seasonal variation, several basic behaviors (e.g. activity, exploration, aggressiveness), and human specific responses between urban and rural habitats.

· I found that urban squirrels have a shorter flight distance and smaller seasonal variation compared to rural squirrels. At the same time, while urban squirrels decreased vigilance to broader risky conditions, they are able to modulate their behaviors in response to different risk levels. Especially, urban individuals exhibited remarkable reduction of flight distance towards human approach. In addition, urban squirrel showed high aggressiveness to human. On the other hand, contrary to general predictions, other behaviors such as activity, exploration, and aggressiveness were not different between two habitats.

· The results suggest that squirrels are not necessarily modifying their fundamental characteristics; rather, human-related behavioral changes would be a key role in adjustment to urbanization. Eurasian red squirrels would be relatively robust to man-made environments due to behavioral flexibility. This thesis highlighted the importance of considering the direct effect of human and deepened knowledge for the process of behavioral modification towards urbanization.

CHAPTER 1

General Introduction



Many of us are living in man-made environments; e.g. currently more than fifty percent of people globally live in urban areas (Grimm et al. 2008; UN 2015). Although urban areas only cover 2.8% of the earth's terrestrial surface (UN 2015), recent rapid extensions of urban areas, termed "urbanization", causes substantial and dramatic environmental disturbance, including forest fragmentation, heat island effect, industrial noise, and light pollution (McDonnell & Pickett, 1990; McKinney, 2002; Gaston 2010). These intensive environmental modifications have imposed novel challenges to many animals. As a consequence, urbanization is one of the main causes of local population extinction and ecosystem destruction, resulting in global scale biodiversity loss (Goddard et al. 2010). One of our missions is to understand the effects of anthropogenic disturbance on biodiversity and minimize its impact on ecosystems at local and global scales.

Animals in an urbanized world

Historically, urban areas were rarely considered as habitat for animals, because urban environments are far removed from natural environments in terms of a high density of humans, buildings, roads, artificial light, noise, and chemicals. Nevertheless, while many species are faced with extinction due to urbanization, some species manage to cope with or adjust to urban environments. For example, raccoons (*Procyon lotor*), red foxes (*Vulpes vulpes*), Eurasian badgers (*Meles meles*), grey squirrels (*Sciurus callorinensis*),

black birds (*Turdus merula*), and house sparrows (*Passer domesticus*) can be seen in urban areas (Adams 2016). Some of such species have shown much higher survival and fecundity compared to conspecifics in natural environments (Ditchkoff et al. 2006; Galbreath et al., 2014). Indeed, urban environments can be characterized as a low predation area due to the decreased number of natural predators, having stable abundant foods due to artificial feeding, and stable local environmental features because it is highly managed by humans (McKinney 2002). In addition, there are some green spaces (e.g. botanic gardens, parks, golf course, and cemeteries) in urban areas, which likely have some potential to provide resources for animals. Urban environments therefore could become an alternative habitat for such urban exploiter species. Investigating how urban animals deal with novel environmental challenges would provide us the insights into adaptation and adjustment of wildlife towards anthropogenic disturbance.

Behavioral adjustment is one of the rapid responses of animals towards environmental changes and recognized as crucial determinants for successful establishment in urban environments. Actually, there is a growing body of literature documenting behavioral modifications in many animals in response to urbanization: shifting foraging behavior, phenology, and personality (Yeh & Price, 2004; Sih et al. 2011; Lowry et al. 2013). Thus, exploring how animals change their behaviors would

reflect impacts of urbanization. For instance, increased boldness (individuals are willing to take more risk) is one of the most well reported behavioral modifications (e.g. Atwell et al. 2012), which is likely due to either the released from predation risk or habituation to humans (Møller 2008; Bateman & Fleming 2014). Moreover, higher levels of exploration and neophilia are also well observed behavioral tendencies in urban animals, because such behavioral traits are advantageous in utilizing human-induced novel resources (Miranda et al. 2009).

Humans are assumed to be one of the most frightful predators for most animals in natural habitats (i.e. super predator, Darimont et al. 2015). Humans kill prey animals at a rate that is as much as 15 times higher than the natural predators in both terrestrial and aquatic ecosystems (Darimont et al. 2015). Therefore, how animals adjust their behavior and physiology could be a key factor to colonize man-made environments as well as urban areas (Blumstein 2016). Because the presence of humans is the main feature of urban environments, urban areas are an ideal environment for detecting direct effects of humans on animal's behavior. People in urban areas exhibit harmless actions and sometimes have affiliated relationships with animals such as providing food. Hence, it could be adaptive for animals to reduce their fearfulness to humans in order to minimize costs associated with being sensitive, leading to habituation to humans (Samia et al. 2015). On the other

hand, this decreased fearfulness to humans could also include potential negative effects. If decreased fearfulness is mirrored in their response to their natural predators because of cognitive constraint, habituation to human may result in increased vulnerability of predation (Geffroy et al. 2015). In addition, close human-wildlife proximity may increase the risk of disease transmissions (Bradley & Altizer 2008) and traffic accidents, which are related to our society's health and economy (Adams 2016). Thus, understanding animal's reaction to humans has raised a big concern among conservation and management researchers (Blumstein 2016; Carrete et al. 2016). The effects of human activity are not only a concern in urban areas, but also in other environments such as agriculture landscapes and natural areas with ecotourism (Blumstein et al. 2018). Therefore, studying such processes and the ecological consequences of behavioral modifications in response to humans could be applied to broader areas of human-wildlife conflicts (Greggor et al. 2016).

A large number of previous studies have revealed behavioral changes, which provided us with general predictions about adaptation and adjustment towards urbanization (Lowry et al. 2013; Sol et al. 2013). However, past studies evaluated only a limited number of behavioral traits in each species and are biased towards birds despite other taxa thriving in urban areas. Thus, assessing various behavioral responses

simultaneously is still lacking. This causes a lack of true understanding of animals' response to the urbanization, because behavioral responses towards human-induced environmental changes is often species-specific. In addition, while many urban areas share some environmental features such as high human density and artificial light levels, other aspects are certainly different, such as time since urbanization, historical background, local climates, and social policy (Johnson et al. 2018). In order to develop our knowledge of the effects of urbanization, evaluating various behavioral modifications in non-avian species is required.

Although many previous studies have shown that increased tolerance to humans is one of the general trends of urban adaptation (e.g. Samia et al. 2015), we do not fully understand this habituation-like processes (Blumstein 2016). For example, measuring flight distances such as flight initiation distance (FID; the distance at which target individuals initiate to fleeing from approaching threats) is the most common method to quantify individual's tolerance towards humans (Blumstein 2016). However, although FID reflects two confounding factors such as individual vigilance (boldness towards broader risky conditions) and risk assessment (as well as habituation), these two factors have not been clearly differentiated. Therefore, the underlying mechanism of increased tolerance to human is still unknown. Animals in urban areas are predicted to interact with

humans more often as compared to other ecosystems. Therefore, urban environments can be regarded as informative field systems to seek and understanding of the processes and consequences of the behavioral response to humans.

Arboreal squirrels should be a model organism

The Eurasian red squirrel (*Sciurus vulgaris*; hereafter, red squirrel) is a typical urban adapter species and is distributed across a wide range of the Eurasian continent from Western Europe to Hokkaido, Northern Japan (Thorington et al. 2013). Originally, they inhabited forest ecosystems, but recently have become common in urban areas (Fey et al. 2015). Red squirrels reside in parks and gardens as alternative habitats in urbanized areas; such habitats are surrounded by man-made structures such as houses and roads, which continuously expose squirrels to anthropogenic disturbances. Because red squirrels are popular with people and sometimes regarded as iconic species of coexistence between human and wildlife, they frequently interact with humans, resulting in decreased flight distances (Uchida et al. 2016). Under the natural conditions, this species is a prey for many predators such as middle size raptors. While they tend to be released from natural predation risks in urban areas, they are also suffering from novel threats such as domestic cats and vehicles (Magris & Gurnell 2002). The red squirrel is relatively easy to observe and capture, enabling us to carry out behavioral experiments and monitor individuals

across time. For these reasons, red squirrels are suitable for exploring the effects of urbanization and human presence.

The main aim of this thesis is to clarify the behavioral adjustments of Eurasian red squirrels to urban environments. I assessed various behaviors including response to humans as compared between urban and rural areas to detect the effects of urbanization. First, I examined the patterns of behavioral responses towards urbanization, and then specifically evaluated the behavioral response towards humans. The three questions examined are as follows;

(1) Does urbanization modify flight distance and its seasonal variation of squirrels?

Changing in anti-predator behaviors has been found in many animals living in urban areas, which may either because of low predation risk and habituation to humans. In addition, phenology and behavioral seasonality also tend to be reduced due to the reduced environmental seasonal variations. Although antipredator behaviors vary seasonally and individual conditions in natural environments, how urbanization modifies this has been overlooked. Therefore, I evaluated flight distance and its seasonal variation of urban and rural squirrels

(2) Next, I evaluated the effects of urbanization on squirrels by comparing several

fundamental behaviors including the response to humans. Generally, past studies showed that urbanization enhances boldness, exploration, and aggressiveness in many species. However, most studies assessed a limited number of behaviors, and often utilized their own unique methods. In this study, I used an open field test (OFT) and mirror image stimulation (MIS), both of which are well-established methods to quantify behaviors and personality traits (Reale et al. 2008). In addition, I also compared boldness and aggressiveness to humans by measuring vertical escape distance (VED) and mobbing call, in order to evaluate how urbanization modifies response of animals to humans

(3) Finally, I investigated the effect of urbanization on boldness and antipredator recognition, particularly differentiating the response to humans and other threats. Antipredator responses could be related to individual survival (Lind & Cresswell 2005). Thus, looking at these behavioral changes could provide us with important information with implications for conservation (Stankowich 2008). In this study, I attempted to differentiate two confounding factors: decreased vigilance and risk assessment (habituation to human). I proposed a simple framework to separate the two underlying processes using alert distance (AD) and flight initiation distance (FID) towards different risk conditions (human, predator, novel object).

Finally, I discuss the behavioral adjustment of animals in response to urbanization and the importance of investigating animal behaviors in this urbanized world from the perspective of conservation and management.

The study area

In this doctoral thesis, I carried out field surveys in the Tokachi region, Hokkaido, Japan. Obihiro city, in which the population is around 160,000, is located in the center of the Tokachi region, with several smaller cities are located around the periphery of Obihiro city. This region was first cultivated 120 years ago. Since agriculture is the main industry in the Tokachi region, urban areas are surrounded by large agricultural farm lands. Substantial forests remain in rural area, and many species including endanger species can be seen in these remaining natural forests.

Eurasian red squirrels inhabit both urban parks and rural forests. In this study, I defined parks as those surrounded by artificial infrastructure and located inside of the urbanization promoting areas (which is used for zoning for urban planning) as urban sites (Figure 1). Rural sites are defined as forest patches in agricultural land that are not surrounded by human residential areas. The year-round artificial feeding by feeding box

or direct feeding can be seen in all urban sites. On the other hand, humans have rarely been seen in the rural sites, and there is no artificial feeding. The main predators of squirrels are the red fox (*Vulpes vulpes*), sable (*Martes zibellina*), and small or middle-sized raptors (e.g. Eurasian hobby *Falco subbuteo*, Goshawk *Accipiter gentilis*, and Sparrowhawk *A. nisus*), and all predators were seen in each rural site. These predators are absent or very rare in urban sites. Domestic cats are also very rare probably due to the harshness of winter.

CHAPTER 2

Seasonal variation of flight initiation distance in Eurasian red squirrels
in urban versus rural habitat



Abstract

Urbanization has caused significant behavioral modifications in wild animals. Change in anti-predator behavior is the most widespread example across different taxa in urban areas, which is probably due to a decrease in predation pressure and habituation towards humans. Seasonality or phenology has also been modified by urbanization since some resources in urban environments are highly controlled, for example, artificial feeding. Under natural conditions, anti-predator responses vary with seasonal variability in environmental and individual conditions. However, resource stability possibly reduces the seasonality of anti-predator behaviors in urban animals. Here, I compare the seasonal difference of flight initiation distance (FID), a measurement of anti-predator response, in Eurasian red squirrels *Sciurus vulgaris* between urban and rural areas in the Tokachi region, Hokkaido, Japan. Rural squirrels possessed FIDs two to three times longer than those of urban squirrels. I also found squirrels in rural areas lowered FID in autumn, but no seasonal difference was observed in urban squirrels. Our results suggest that continuous supplementary feeding may have buffered the seasonality in anti-predator response. In addition, strong habituation to humans may allow urban red squirrels to correctly assess human activity as benign rather than reacting unnecessarily.

Introduction

Recent rapid urbanization has substantially affected many wild animals via extreme anthropogenic disturbance, such as habitat loss and fragmentation, road strike, industrial noise, and light pollution (McDonnell & Pickett, 1990; McKinney, 2002; Bateman & Fleming, 2012). While urbanization is one of the main causes of population extinction (Goddard, Dougill & Benton, 2010), some animal species have managed to cope with, or even adapted to living in cities (Ditchkoff, Saalfeld & Gibson, 2006; Galbreath *et al.*, 2014). Such animals show significant modification in behavior and life history when compared to rural conspecifics, often differing in foraging behavior, phenology, and personality (Yeh & Price, 2004; Sih, Ferrari & Harris, 2011; Lowry, Lill & Wong, 2013). These modifications may reduce anthropogenic stresses and enable animals to better utilize resources in urban areas.

One of the most remarkable behavioral modifications urban animals display is the changing of anti-predator responses (Lowry *et al.*, 2013). Animals require high vigilance levels or sensitivity to predation stimuli, which incurs some costs on foraging or energy intake (Cooper & Pérez-Mellado, 2004). In urban environments however, the abundance of natural predators is generally decreased (Fischer, Cleeton & Lyons, 2012) and animals no longer need to maintain high levels of vigilance. Urban animals, therefore, become

bolder and less vigilant than their rural counterparts, which is often interpreted as an adaptive response (Møller, 2008). In addition, changes in anti-predator behaviors may be strongly modified by human presence (McCleery, 2009; Engelhardt & Weladji, 2011; Bateman & Fleming, 2014). Although humans are potential predator for some species, in urban environments we generally do not display much concern towards harmless animals present and even feed them on occasion. Urban animals are thus continuously exposed to non-lethal stimuli, resulting in a reduction of responsiveness to human approach (McCleery, 2009). Such habituation to humans may decrease anti-predator responses even towards other predators (Lowry *et al.*, 2013).

Seasonality or phenology has also been modified in some urban animals (Shochat *et al.*, 2006). City parks and private gardens are important habitats for urban animals, and resources in such habitats (e.g. vegetation, rivers and ponds) are relatively abundant and stable owing to human control (Shochat *et al.*, 2006). Accordingly, urban animals are able to acquire resources throughout the year, which alters the seasonality of the environment in urban areas (Bateman & Fleming, 2012). For example, artificial feeding strongly affects migratory birds, so that some individuals become sedentary (Jokimäki *et al.*, 2002). In addition, the increase of residents in urban areas has, in turn, reduced seasonal differences in population dynamics and community structures (Shochat, 2004; Murgui,

2007; Leveau & Leveau, 2012). Buffering seasonal variation in behaviour may be an important modification that potentially influences biota in urban areas.

Seasonality in anti-predator responses could also be modified in urban areas. Under natural conditions, anti-predator responses would be high during the breeding season or while caring for offspring since they suffer higher predation risk for offspring and themselves (Ciuti *et al.*, 2008). Conversely, during the seasons when high energy input is needed, such as the growing season or before wintering, animals pay heavier costs for foraging than anti-predator behavior (Barnard, 1980). In addition, there may also be other environmental factors that can potentially affect seasonality in anti-predator response, such as temperature and seasonally fluctuating resources (Rand, 1964; Manor & Saltz, 2005; Stankowich & Blumstein *et al.*, 2005; Stone, 2007). In urban areas, predation pressure and/or resources availability should be relatively stable (Shochat *et al.*, 2004) and therefore the seasonality of anti-predator responses may be buffered.

Flight initiation distance (FID) is one of the most useful measurements for anti-predator response (Stankowich & Blumstein, 2005). FID is the distance from approaching predators at which prey flees and allows us to quantify the degree of disturbance posed by potential predators towards prey animals (Ydenberg & Dill, 1986). Due to the relative ease of measuring in the field, studies using FID have been accumulating over the past

few decades and thus provide us both an empirical and theoretical base (Ydenberg & Dill, 1986; Blumstein, 2006; Cooper & Frederick, 2007). We can develop specific testable predictions for FID in urban animals and its seasonality based on the optimal escape theory (Figure 1; Ydenberg & Dill, 1986; Cooper & Frederick, 2007). First, FID should be reduced in urban areas compared to rural areas. Flight decisions are predicted to be based on the balance of cost due to remaining (e.g. predation risk; curved lines in Fig. 1) and the cost due to fleeing (e.g. loss of foods, opportunity cost; linear lines in Fig.1), and prey initiate flight when the cost of fleeing is outweighed by the cost of remaining (point of the intercept of curved and linear lines in Fig.1). In urban areas, since the risk of predation and mortality due to pedestrians is low (McCleery *et al.*, 2008), the cost of remaining when approached by a person would be decreased (Fig.1). In addition, since artificial feeding (e.g. feeding boxes) is attractive and an important resource for urban animals (Shochat *et al.*, 2006), the opportunity cost of fleeing would be increased (Fig.1, Cooper & Frederick, 2007). Therefore, optimal FID would shrink when compared to more natural conditions found in rural areas, a prediction that is supported by many past studies (McCleery, 2009; Engelhardt & Weladji, 2011). Second, FID should change seasonally under natural conditions (Figure 2a, Stankowich, 2008, Cooper, 2009). During the breeding season or while caring for offspring, the cost of remaining would be

increased (i.e. dashed lines in Figure 2a), and FID also tends to increase (Ciuti *et al.*, 2008). On the other hand, during the seasons when energy requirements are high, such as before wintering, the opportunity cost would be high (i.e. solid lines in Figure 2a), and therefore FID would decrease (Barnard, 1980; Cooper & Frederick, 2007). By contrast, environmental conditions are comparatively stable in urban areas, especially when artificial feeding is ongoing (Shochat *et al.*, 2006), and therefore the seasonal differences in lost opportunity costs or differences in energy requirements would be minimal. Therefore, seasonal variation in FID should be reduced in urban areas (Fig. 2), a hypothesis that has not yet been tested. Natural predators also tend to be rare in urban habitats and humans do not generally change their behavior seasonally toward animals. Such relative consistency across seasons may modify anti-predator behavior only slightly, although other potential factors, such as physiological and metabolic change due to seasonality, may still apply.

To test these predictions, I compared the seasonal patterns of FID in the Eurasian red squirrels *Sciurus vulgaris* between urban and rural areas in the Tokachi region, central Hokkaido, Japan. Arboreal squirrels, such as red squirrels *S. vulgaris*, fox squirrel *S. miger*, and gray squirrels *S. carolinensis* are well adapted to urban areas, thus are suitable species for examining the effects of urbanization (Lee & Fukuda, 1999; Mcleery, 2009;

Thorington *et al.*, 2012). Squirrels can be found close to humans in Obihiro's city parks where predators are scarce and squirrels often depend on artificial feeding provided by citizen throughout the year. Under these conditions, red squirrels in urban areas should display a shorter FID than conspecifics in rural areas, and our main prediction is that urban red squirrels show smaller seasonal variation in FID as described above.

Materials and Methods

Study area and species

Research was conducted over all four seasons during 2014 in the Tokachi region, central Hokkaido, Japan (Fig.3). This area is a large floodplain system of the Tokachi River, the sixth largest watershed in Japan (9 010 km²) and has been highly cultivated for agricultural use since 1883 (Akasaka, Akasaka & Yanagawa, 2010). The relatively large city of Obihiro (population of roughly 160,000) is located in the middle of this watershed, and is surrounded by rural agricultural land. A large number of fragmented forests are present in this region. Seasonal variations of the environment are relatively large: air temperature often exceeds 30°C in summer and falls below -20°C in winter. Annual precipitation averages 887.8 mm and more than 2 m of snowfall are recorded every winter. I selected a total of 12 fragmented forests (ca. 0.9 – 59.5 ha) as study sites, of which half

were urban sites while the other half were rural sites (Fig. 3). All sites were more or less isolated (at least 1km apart from the nearest habitat patches). Considering the short time scale of our experiment (< four weeks in each survey), I believe that the same individuals were not observed at different sites (especially between rural and urban sites). I selected city parks and the campus of Obihiro University of Agriculture and Veterinary Medicine as urban sites, whereas isolated forests near mountains and those in agricultural lands were selected as rural sites. The urban and rural sites primarily differed in human density, infrastructures (e.g. houses, buildings, roads), and traffic levels. Each urban habitat contained a recreational playground or pathway and was surrounded by dense residential areas and wide roads. Each rural site was fragmented forest without man-made structures and was surrounded by agricultural lands. Live Korean pine *Pinus koraiensis* and Manchurian walnut *Juglans mandshurica* var. *sachalinensis*, the main diet of local red squirrels, were present both in urban and rural sites. I observed feeding boxes or direct feeding by humans throughout the year in all the urban sites, but never in the rural sites. Underbrush in most urban sites was well managed and cut lower than the height of red squirrels while they stand, whereas bushes or shrubs grew to about 0.7 - 1.5 m in height from early summer to early autumn in rural sites, which made the field survey difficult in some seasons (see below). Foxes and small or middle-sized raptors (e.g. Eurasian hobby

Falco subbuteo, goshawk *Accipiter gentils*, sparrowhawk *A. nisus*), the main predators of red squirrels, were rarely observed in urban sites and also the number of domestic cats was remarkably small in Obihiro city, whereas in rural areas at least one predator was observed in every site.

Eurasian red squirrels *S. vulgaris* are widely distributed across northern Eurasia (i.e. from Europe to Hokkaido, Japan). As in European populations, red squirrels in Hokkaido are commonly observed even in small parks or fragmented forests in city centers (K. Uchida, pers. obs.). Although the diet of red squirrels is diverse (e.g. seedlings, mushrooms, insects), they rely strongly on tree seeds, such as walnuts and coniferous seeds (Wauters *et al.*, 2001a, b). They hoard the seeds within their home range for winter or the next spring when energy rich foods are no longer available (Wauters, Suhonen & Dhondt, 1995). Because the hoarding behaviour can significantly affect winter survival, squirrels concentrate on hoarding activity in autumn (Wauters *et al.*, 1995). In spring, red squirrels engage in mating and nursing (Wauters & Dhondt, 1989), whereas activity is relatively low during summer and winter. Therefore, I predict that FID is short in autumn and long in spring in rural sites, whereas such seasonal variation will be reduced in urban areas (Fig. 2).

Measurement of FID

Data was collected in the winter (3-9 February), spring (5-30 March), summer (30 July to 4 August), and autumn (24 September to 30 October) from each study site. Observations took place during red squirrel high activity periods (from sunrise to 10:00 a.m.). I mainly focused on spring and autumn for comparisons between urban and rural areas, since I could not collect enough data in rural areas during summer and winter due to thick underbrush and the low activity of squirrels in each season respectively. On the other hand, all seasons were used for seasonal variation in urban areas. I visited each site randomly and at least twice each season.

Following Dill & Houtman (1989), FID was measured from each individual found during a line census. When I found a squirrel foraging on the ground, the observer approached the squirrel directly at a constant pace of 0.5 m/s. When the subject fled, the distance between the observer and subject was measured (to the nearest 0.1 m). Since the start distance possibly affects FID (Rodriguez-Prieto *et al.*, 2009), I measured FID only when a squirrel was found more than 40 m away from the observer. To avoid resampling from the same individual at each line census I tried to remember the direction they fled, behaviors after fleeing (e.g. foraging, staying, moving), and distinguishing features of individuals (e.g. body size, condition, and coat colour) and did not collect data whenever

there was a possibility of resampling. I also tried to minimize potential sampling biases: for example, I did not measure FID (1) when squirrels detected the observer before initiation of approach, (2) when there were conspecifics nearby (i.e. targeted solo squirrel only), (3) when I heard alarm calls from conspecifics or some avian species such as Eurasian jays *Garrulus glandarius*, (4) when pedestrians or bicycles crossed between the observer and squirrel, and (5) when squirrels approached the observer, possibly begging for food. In addition, to reduce the effects of noise and the observer's bias on FID, censuses were mainly conducted by a single person (K. Uchida) with occasional help from another person.

I recorded local environmental factors at the point each squirrel was initially found (i.e. focal point), distance to the tree that the target squirrel climbed for fleeing (m), canopy coverage (%), and vegetation height (cm), which all possibly affect FID (Blumstein, 2006; Engelhardt & Weladji, 2011). Canopy coverage (%) was measured by visual observations based on the method used by Freites, Cerqueira & Vieira, (2002). Vegetation height was measured from the average of three randomly chosen points within the radius of 2 m from the focal point.

Statistical analysis

To assess if FID was affected by area, season, and local environmental factors, I used a linear mixed model (LMM) with the area (urban or rural), season, (spring or autumn), interaction of area and season, distance to tree, canopy coverage, and vegetation height as fixed effects, and the site ID as a random effect. Both area and season were used as categorical variables. I also used a similar LMM to investigate the difference in FID among the four seasons in urban squirrels. FID was included as a dependent variable, season, distance to tree, canopy coverage, and vegetation height as fixed effects, and the site ID as a random effect. Since the season has four categories (i.e. winter, spring, summer, autumn), I used binary dummy variables setting winter as a standard. Multicollinearity among the candidate variables was assessed using the variance inflation factor (VIF) and was not detected (i.e., $VIF < 2.0$). To perform the LMMs I used statistical software R (version 3.0.2).

Results

A total of 237 FIDs was measured (urban area: winter $n = 18$, spring $n = 51$, summer $n = 18$, autumn $n = 85$, rural area: spring $n = 31$, autumn $n = 34$). For comparison between urban and rural areas and between seasons, FID was significantly affected by area, season, and the interaction, whereas local factors did not significantly affect (LMM; Fig.4; Table

1). FID of urban red squirrels was half to a third the distance of rural squirrels regardless of the season (Fig.4). In rural areas, FID was shorter in autumn than in spring whereas there were no clear differences in FID between autumn and spring in urban sites (Fig.5). No seasonal difference in FID in the urban areas was also supported by the LMM across four seasons (Fig.5; Table 2). FID was consistently small (range: 2 - 8 m) in urban squirrels throughout the year.

Discussion

To date, reductions in flight responses have been well documented in urban animals, and the buffering of behavioral seasonality is also found in some avian species (Shochat *et al.*, 2006; Murgui, 2007; Leveau & Leveau, 2012). However, as far as I know, this is the first study suggesting that urbanization can also decrease the seasonal variation in anti-predator response. I did not find any significant effects with regards to local factors that potentially affect FID. Although the lack of the significance might be merely due to relatively limited sample size, our results clearly indicate that urban-rural differences have stronger effects on FID and its seasonal patterns. This behavioral modification may be one of the adaptive responses in wild animals to urbanization.

As predicted, I was able to approach urban red squirrels much closer than rural

individuals, which is consistent with other studies on arboreal squirrels (Cooper *et al.*, 2008; Mccleery, 2009). Squirrels are sometimes considered as an iconic species for “happy coexistence” between humans and wildlife, since they are common in the parks of many metropolitan cities and appear adjusted to an urban lifestyle (Thorington *et al.*, 2012). However, the results of this study showed that rural squirrels were innately highly sensitive to human activity. The FID value in rural areas was long (average 19 m) but still underestimated; I often failed to collect FID in rural areas due to squirrels noticing the observer earlier than I noticed the squirrel, running away at distances greater than 40 m. This implies that squirrels are naturally sensitive (this is understandable since squirrels are a major prey species for many animals, Randler, 2006), whereas urbanization can significantly modify anti-predator behavior, possibly due to the release from natural predators, habituation to humans, and strong reliance on artificial feeding (Fig. 1).

Does the reduction of FID in urban squirrels mean that they lose vigilance or become bolder when compared with rural conspecifics? The answer is not straightforward, because FID not only reflects vigilance (Fernández-Juricic & Schroeder, 2003; Blumstein *et al.*, 2005) but also includes risk assessment (Stankowich & Blumstein, 2005; Stankowich, 2008). Thus, urban squirrels might have been aware of the observer but accepting of their presence until a close proximity since they might see humans as low

risk (i.e. vigilance does not decrease but FID does). In fact, urban grey squirrels can differently respond to different behavior of pedestrians, according to different levels of threat posed to them (Bateman & Fleming, 2014). Therefore, selection on personality traits is not necessary for explaining the reduction of FID in urban environments: flexible behavioral changes based on risk assessments may allow the squirrels to succeed in the city life. Separating the two mechanisms (i.e. adaptation vs. plasticity) is particularly important because, although natural predators decrease in urban areas, meso-predators such as domestic cats and dogs, become an alternative danger for urban animals (Prugh *et al.*, 2009; Valcarcel & Fernández-Juricic, 2009). If the reduction of FID reflects the loss of vigilance, such urban animals may not correctly respond to novel predators.

Rural squirrels modified their FID in response to seasons in the direction consistent with our initial predictions that FID increases in spring (i.e. breeding season) and decreases in autumn (i.e. high foods requirements for winter). I could not collect data during winter in rural area, which is also an indicative of seasonal variation in activity levels. It was difficult to find squirrels during winter, even though their footprints were present on the snow and the view was better than other seasons due to less vegetation and fewer leaves on trees. Squirrels would reduce activity during winter to save energy (Humphries *et al.*, 2005) and potentially to also avoid predators. Or, they might have fled

from a much further distance (> 40 m), so I could not have observed them: good visibility and the long distance sounds carry in winter might allow squirrels to detect potential threats at longer distances. Seasonality in FID has received little attention but is worth investigating to better understand anti-predator and optimal foraging behavior.

Given the results in rural areas, no significant seasonal difference of FID in urban squirrel was a little surprising: FID was consistently short (5-6 m) even in winter. This suggests that the proposed factors (i.e. release from predators, human habituation, consistent supplementary feeding; Figure 2b) strongly affect risk assessment and outweigh even seasonally changing natural conditions. Among the candidate factors, habituation to humans might be most influential since squirrels have a good ability to recognize different risk levels in humans (Bateman & Fleming, 2014). Future research should separate the effect of each factor, and experimental studies such as presenting novel objects and manipulating food abundance would merit further attention to identify the main causes of the reduction in seasonality of FID in urban sites.

Figure legends

Figure 1. Graphic model of optimal flight initiation distance (FID) in rural (natural) and urban areas (modified from Ydenberg & Dill 1986, see details in the text). Two curved lines represent cost of remaining, such as predation, which decreases with increasing distance, whereas two linear lines represent cost of fleeing, such as loss of foraging opportunity, which increases with increasing distance. The intersection between the curved and linear lines should represent the optimal FID. Solid lines indicate the cost dynamics in urban areas and dashed lines indicate cost dynamics in rural areas. The cost of remaining should be decreased in urban areas due to the release from predation and human habituation. The cost of fleeing should be increased in urban areas due to the strong reliance on artificial feeding.

Figure 2. Seasonal changes in optimal flight initiation distance (FID) in (a) rural and (b) urban areas. Refer to Figure 1 for the meaning of the lines and intersections. For simplicity we describe only two seasons (spring and autumn) and are designed for the study species, red squirrels. In rural areas (natural habitat), cost of remaining would be high in spring (breeding season) and cost of fleeing would be high in autumn (before winter), which makes FID shorter in autumn compared to spring. In urban areas, similar seasonal changes should be held but the variation becomes smaller due to stable environmental conditions, such as artificial feeding throughout the year, compared to rural areas. See details in the text.

Figure 3. Seasonal patterns of FID in urban and rural red squirrels. Means and standard deviations are shown. Sample size of urban squirrels is 136 (autumn = 85, spring = 51) and rural squirrels is 65 (autumn = 34, spring = 31).

Figure 4. Seasonal patterns of FID in urban red squirrels. Means and standard deviations are shown. No significant difference was observed among the four seasons (see text and Table 2 for detail). Sample sizes of each season are: winter = 18, spring = 51, summer = 18, and autumn = 85.

Table.1. Result of linear mixed model (LMM) of FID, comparing between urban and rural red squirrels and between spring and autumn and local factors (distance form tree, canopy coverage and vegetation height).

	Estimate	S.E.	d.f.	<i>T</i>	<i>P</i> -value
Intercept	16.059	2.240	46	7.168	<0.001
Area (urban/rural)	-9.513	2.278	18	-4.175	<0.001
Season (autumn/spring)	4.886	2.168	152	2.254	<0.026
Area × Season	-6.083	2.625	153	-2.318	0.022
Distance from tree	0.069	0.135	150	0.510	0.611
Canopy coverage	0.138	0.021	153	0.671	0.503

Vegetation height	0.217	0.137	153	-2.318	0.114
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Table.2. Result of linear mixed model (LMM) for the FID of urban red squirrels among four seasons. Winter, during FID is lowest, is set as a standard in this analysis and compared with other seasons and also the effects of local factors (distance form tree, canopy coverage and vegetation height).

	Estimate	S.E.	d.f.	<i>T</i>	<i>P</i> -value
Intercept	5.175	1.992	16	2.598	0.020
Season (winter)					
Spring	-0.216	1.762	138	-0.123	0.902
Summer	-0.534	2.275	138	-0.235	0.815
Autumn	0.599	1.550	137	0.387	0.700
Distance from tree	0.093	0.115	137	0.818	0.415
Canopy coverage	0.030	0.018	137	1.735	0.085
Vegetation height	-0.190	0.275	139	-0.692	0.490

Fig.1

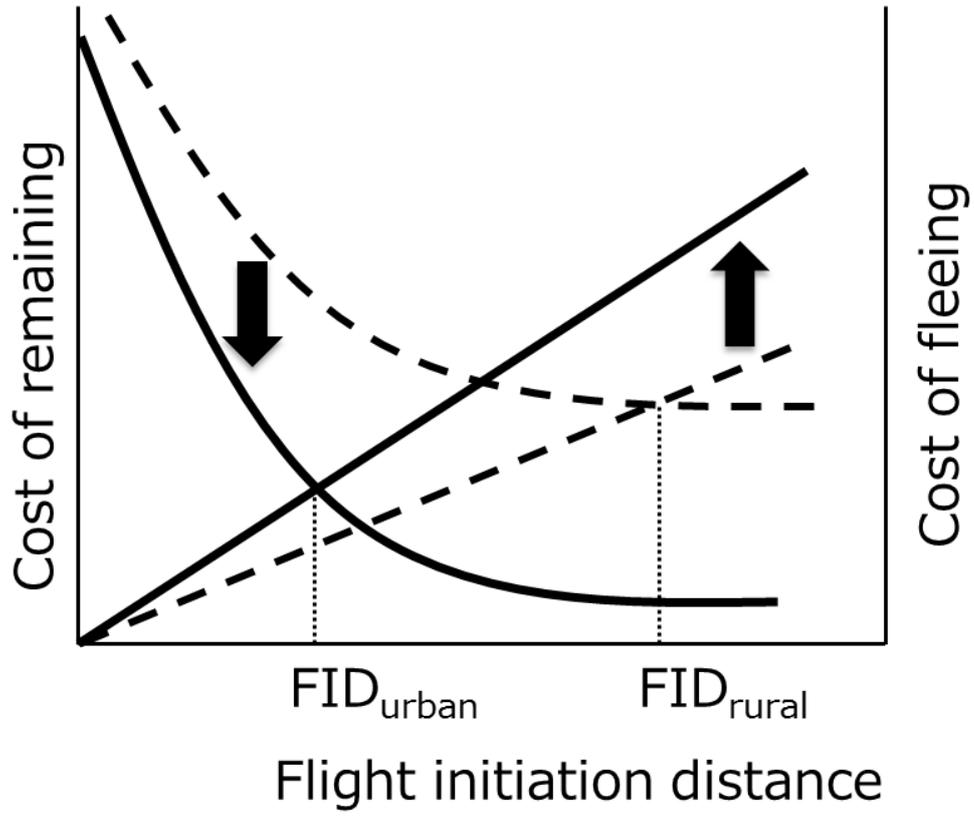


Fig.2

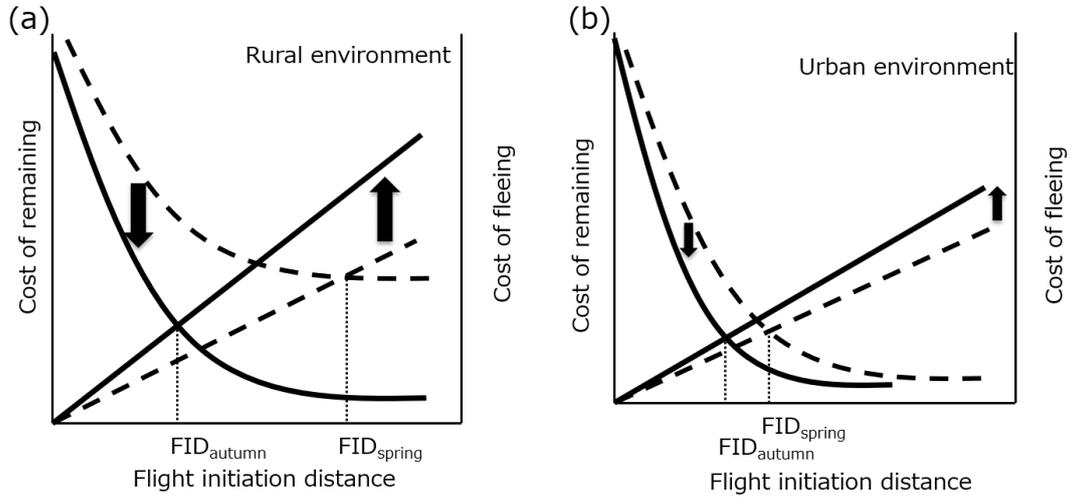


Fig.3

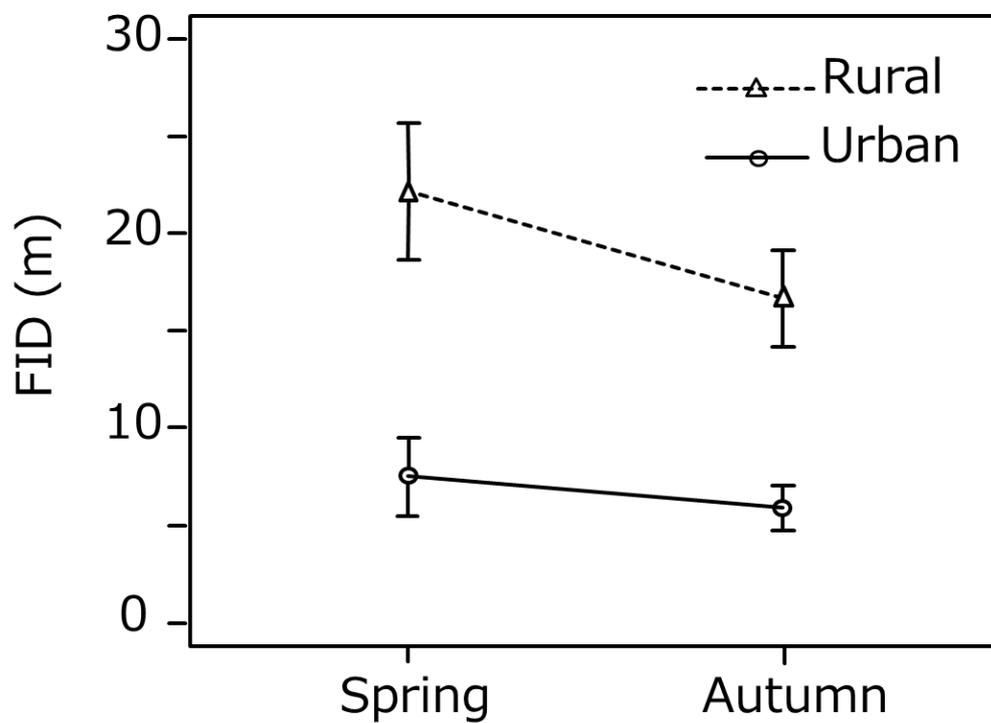
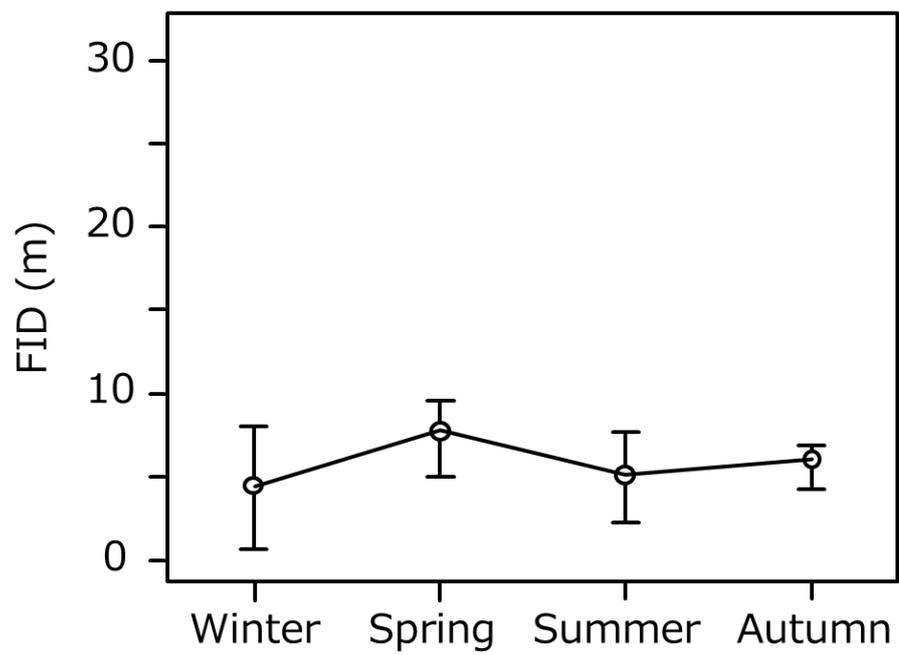


Fig.4



CHAPTER 3

No difference in various behaviors between urban and rural squirrels except for human-induced response



Abstract

Over the last decades, many studies have reported that animals living in urban environments modify behaviors to cope with anthropogenic disturbance. In general, as urbanization increases, animals become bolder, more explorative, and more aggressive compared to natural conspecifics. However, past studies were biased toward birds and limited in the number of behavioral traits examined, often employing species-specific unique methods. In this study, I compared multiple behaviors between urban and rural Eurasian red squirrels *Sciurus vulgaris* using the open field test (OFT) and mirror image stimulation (MIS), which are well-established methods in the field of animal behavior or animal psychology. I also used other measurements, such as flight distances and the number of mobbing calls directed at humans. Principle component analysis for OFT and MIS provided some axes, which had repeatability within individuals, but these axes were generally difficult to interpret. I, therefore, examined each behavior separately: contrary to previous studies, no difference was observed between urban and rural individuals in most behaviors, except for human-related behaviors. Behavioral modifications reported in previous studies might reflect only human-related disturbances: urban animals may be able to flexibly change behaviors depending on the situation, while their innate character

remains unchanged. This study underscores the importance of measuring multiple behaviors using different methods, especially focusing on human-associated behaviors.

Introduction

Urban ecology is one of the emerging fields in ecology (Gaston 2010) and recent studies have suggested that animals living in urban areas exhibit different behaviors compared to conspecifics in natural habitats (Ditchoff et al. 2006; Sol et al. 2013). Increased boldness, exploration, and aggression are the most widely reported behavioral shifts in urban environments (Lowry et al. 2013). These characteristics are considered adaptive for exploiting urban resources under predator free environments (Møller 2008): human induced resources, such as garbage and artificial feeding, are sometimes highly abundant and predators generally avoid man-made environments (and humans also eliminate large predators). While general trends in behavioral changes would be valid, the response to urban environments should be species-specific (Birnie-Gauvin et al. 2016) and past studies are highly biased toward birds. More empirical studies are needed especially for non-avian species (e.g., Lyons et al. 2017; Hurtado et al. 2017).

Comparing behaviors between urban and rural (natural) areas is a common approach to investigate how animals adapt/respond to urbanization. Flight distances such as flight initiation distance (FID) is probably the most widely used measurement to examine behavioral change (Lowry et al. 2013). FID is the distance at which target animals flee from an approaching object (usually the observer) and therefore should

reflect boldness (Stankowich & Blumstein 2005; Stankowich & Coss 2005). Because FID is simple and easy to measure in the field, its standardized nature enables comparative analyses among different species (Stankowich & Blumstein 2005; Blumstein 2006). Open-field tests (OFT) and mirror image stimulations (MIS) are also common and standardized methods to evaluate behavioral traits (Svendsen & Kansas 1973; Perals et al. 2017), although these require the capturing and handling of animals. OFT and MIS have long histories initially used around 1930's to quantitatively measure individual behaviors in lab animals, mostly mice and rats (Hall & Ballachey 1932; Walsh & Cummins 1976). Animals are put in a simple box and activity is estimated from the proportion of time in locomotion and distance traveling. Exploratory behavior, curiosity, and neophilia (neophobia) can be also measured by putting a novel object in the box (Réale et al. 2010). MIS is generally employed together with OFT: after collecting OFT data a mirror is set to the box and response to conspecific, such as aggressive and social behaviors, are evaluated (Svendsen & Kansas 1973). More recently, researchers use OFT and MIS to measure a series of different behaviors and extract some sets of behavioral tendencies with a principal component analysis (PCA), in which the axes are often assumed as animal personality (Perals et al. 2017). Other than FID, OFT and MIS, researchers employ specific methods to assess behavioral changes due to the urbanization

for each species. Past studies have examined only a few behavioral characteristics: employing various behaviors is still lacking.

In this study, I compared multiple behaviors of Eurasian red squirrels *Sciurus vulgaris* between urban and rural environments, including flight distance (a modified version of FID), OFT, and MIS. Squirrels are one of the typical urban colonizers seen in many urban areas (Thorington et al. 2013; Fey et al. 2015; Uchida et al. 2016): they seem well adjusted to anthropogenic disturbances. Because squirrels' behaviors are relatively easy to observe in the field and their small sizes allow OFT and MIS, squirrels are a good mammal model to investigate the adaptation to urban environments. In this study, I firstly tested whether PCA approach for OFT and MIS can categorize typical behavioral traits, such as activity, exploration, and aggressiveness, as suggested in previous studies (Martin & Réale 2008). Next, I examined if each behavior showed within individual consistency across time, which is a premise of animal personality (Réale et al. 2007). Then, I compared the behaviors between urban and rural habitats. Finally, I also compared vertical escape distance (VED), which is a modified version of FID for arboreal species (Uchida et al 2017), and the number of mobbing calls as a measurement of aggressiveness between urban and rural squirrels. VED is the height at which a target individual stops climbing the tree to escape from an approaching object: bolder

individuals stop at lower heights (Uchida et al. 2017). I specifically tested the general predictions whether urban individuals are bolder, more explorative and aggressive using the Eurasian red squirrels in the Obihiro city, central Hokkaido, Japan.

Materials and Methods

Field sampling

Data collection was carried out in and around Obihiro city from 2016 to 2018. Obihiro is the largest city (the population size is about 160,000 people) located in the center of the Tokachi plain, which is surrounded by rural agricultural lands. I selected four city parks as urban sites and four forests as rural sites. Rural areas were selected based on being at least 10 kilometers away from urban centers. The urban and rural areas differed in human density, artificial structures (e.g. houses, buildings, roads) and traffic levels. Urban habitats generally contain recreational playgrounds or pathways and are surrounded by dense residential areas and wide roads. On the other hand, rural habitats are surrounded by agricultural lands or next to the mountains. While some forests have a few man-made structures, I rarely encountered other humans, and the forests were not managed (e.g. underbrush was not cut). There is year-round artificial feeding from citizen in all of the urban sites. I have never observed artificial feeding in the rural sites for at least four years

since I started researching on squirrels in these populations.

Red squirrels were live-trapped for marking and behavioral experiments such as OFT and MIS. I conducted trapping in two seasons (spring; May and Jun, autumn; October and November) in each year. Box traps (Model RB-2, Sakae Industry Co., Ltd. Niigata, Japan) were placed on the ground, and pine cones and walnuts were used to bait each trap. I started trapping from sunrise to 1200 h. Soon after squirrels were captured, they were put in an experimental box to measure behaviors. After data collection, I measured body weight and marked each squirrel with unique colored collar and ear tag. In addition to the OFT and MIS, I collected data on VED and the number of mobbing call during a thirty-minutes line census in each site: unmarked squirrels were also recorded for VED and mobbing call. All research procedures were accordance with American Society of Mammalogists (Sikes et al. 2016) and carried out under the permission from Institutional Animal Care and Use Committee of National University Corporation Hokkaido University (15-0121).

Measurement of behaviors

I captured 40 squirrels from urban habitats and 18 from rural habitats (33 adult males and 25 adult females). All squirrels were recaptured at least once and used for OFT and MIS

from two to seven times to test the repeatability within individuals, although I could not standardize the interval (from seven days to one year). I performed OFT for 7.5 minutes and MIS for 5 minutes with a $90 \times 100 \times 70$ cm white box made from acrylic lid and squirrels' behaviors were recorded using a digital video camera (JVC Everio, Yokohama, Japan). The floor of the box was divided into four parts with two grid lines. Four blind holes (3 cm diameter) were located on the floor for measuring exploration behavior. A 60×40 cm mirror was fixed to one side of the box for MIS, which can be covered during OFT: after finishing an OFT trial, the cover was removed and the squirrel's responses to the mirror were recorded. Squirrels' behaviors were obviously changed after exposure to the mirror. I cleaned the box with 70% ethanol after each trial.

During the thirty minutes line census in each site, I measured several flight distances (Uchida et al. 2017). When I found a squirrel, I approached at a constant speed (e.g. 1.0m/s) and measured AD (alert distance), FID, and VED as an indicator of boldness. Since these are generally correlated each other and VED is the most efficient for data collection (Uchida et al. 2017), I used VED in this study. VED is the height at which a target individual stops climbing the tree to escape from an approaching object (Uchida et al. 2017). The distances they climbed were measured by a laser rangefinder (Tru-Pulse 200, Laser Technology Inc., Centennial, Colorado, U.S.). Marked individuals were

measured multiple times at different periods. In total, I collected data for 44 marked individuals from urban habitats and 19 marked individuals from rural habitats (30 adult male and 33 adult female). After data collection of VED, the number of mobbing calls were also measured as an indicator of aggressiveness. Once they climbed trees, they stopped at certain heights (i.e., VED), and then showed several behaviors, such as gazing, alert call, run away, foraging, and mobbing call. Individuals often approached to human with intensive calls and tapping (one of the aggressive behaviors in Eurasian red squirrel; K. Uchida personal observation), which was obviously different from alert call. Soon after I recorded VED, I started to measure the time and count the number of mobbing calls within 30 seconds, when individuals showed the mobbing behavior. I collected a total of 35 mobbing events from 31 urban and 4 rural squirrels. I measured the number of mobbing call not only from marked individuals but also from unmarked squirrels because rural squirrels rarely did mobbing calls and the sample size was small. When I observed unmarked squirrels, I identified individuals from morphological characteristics such as body color and scar to avoid double counting on the same day.

Data analysis

The video data of OFT and MIS were analyzed using “tanaMove software” (version 0.09), which is freely available from the website of the NIG

(<http://www.nig.ac.jp/labs/MGRL/tanaMove.html>). First, I made an ethogram of the red squirrels (Table 1) according to the previous studies that used OFT and MIS for Sciurid squirrels (Boon et al. 2007; Martin & Réale 2008; Petelle & Blumstein 2014). I also made several original behavioral classifications because the study species showed some specific behaviors; for example, our red squirrels showed tapping and tail raising as an aggressive response rather than attacking to the mirror (Boon et al. 2007). As a result, nine and 12 behaviors were described for OFT and MIS, respectively (Table 1). The meanings of some behaviors were reasonably clear: proportion of time locomotion and the number of lines crossed are generally regarded as “activity” (Boon et al. 2007; Martin & Réale 2008), number of head dips to a hole probably reflect “exploration” (Boon et al. 2007), self-grooming reflects “stress response” (Martin & Réale 2008), and attack toward a mirror should be “aggressiveness” (Boon et al. 2007). If each individual has some “personality” PCA would categorize similar behaviors (e.g. locomotion and line cross). The times each squirrel spent for each behavior were calculated with the resolution of 0.25 seconds. Because some behaviors were done simultaneously (e.g. tail raising and gazing the mirror), the total amount of time for each individual analyzed exceeded 7.5 min for OFT or 5 min for MIS. I also counted the numbers of events (e.g. lines crossed, head dipping, jumping and tapping) when applicable (Table 1).

I performed principal components analyses (PCA) for OFT and MIS to see if some of the behaviors (i.e., Table 2,3) are categorized into broader behavioral tendencies, such as activity, exploration, aggressiveness, sociality, and stress response (Martin & Réale 2008). I performed PCA for urban and rural squirrels separately because they might have different behavioral characteristics. I also assessed the repeatability of each PCA axis to test whether each individual show behavioral consistency within individual across times, by which the behavioral character of each individual can be called “personality” (Carere & Maestriperi 2013). I calculated the repeatability using the R package rptR v0.9.2 (number of parametric bootstraps for interval estimation = 5000, number of permutations was = 10000; Santicchia et al. 2018). The linear mixed-effects models (LMM) were constructed with the PCA axis as the dependent variable and with the area, seasons, sex, and their interactions as fixed effects: squirrel ID was also used as a random effect. Gaussian error distributions were given as probability distributions. Statistical significance of the repeatability was tested by likelihood ratio testing (LRT) with and without the random effect (ID), following Bolker et al. (2009). The confidence interval of repeatability was estimated by parametric bootstrapping. Statistical significance towards H_0 , and the zero repeatability was testing based on the likelihood ratio and the permutation analysis.

I also performed generalized linear mixed-effects models (GLMM) to investigate the difference of each behavior (i.e., Table 1) between urban and rural areas. Because the results of PCA were often difficult to interpret (see Results), I decided to compare each behavior separately. I selected the behaviors that are relatively straightforward to interpret (e.g., T locomotion, T look, T groom, and N lines, N head dips; details are shown in Table 1). Time proportions of each behavior in OFT, MIS and metric data of VED were included as dependent variables with normal distributions, while count data were included as dependent variables with Poisson distributions. The area (urban or rural), season (spring or autumn), sex and interactions of each variable (area \times season, season \times sex, area \times sex) were included as independent variables, and individual ID as a random effect. For analyzing the number of mobbing calls I employed a hurdle model because some squirrels exhibited mobbing behavior while others not: the number of mobbing calls was zero for the latter. The hurdle model is a two-component hierarchical model, including logistic regression for zero data and a generalized linear model for count data (Dicken and Booth 2013). Therefore, I can evaluate whether squirrels exhibited mobbing behavior with the logistic regression model, and the number of mobbing calls with the generalized linear model. I included the area (urban or rural), season (spring or autumn), sex and their

interactions as independent variables. All data were analyzed using the statistical program R (R Development Core Team, 2012)

Results

Behavioral traits using OFT and MIS

For the PCA analysis of OFT, PC1 to PC3 covered 76.4% of the total variance in urban areas and 67.1% in rural areas (Table 2, 3). PC1 of both areas were similar and can be categorized as activity because this axis included “N line”, “T locomotion”, and “N jump” (Table 2,3). PC2 from urban and rural squirrels were little different: for example, “N head dipping”, the most typical explorative behavior (Martin & Réale 2008), was included only in rural areas. In addition, this axis is difficult to interpret because this included both explorative behaviors (“T stand look” and “T scanning”) and stressed behaviors (“T grooming”) (Table 2,3). PC3 also showed similar tendency with PC2, including both explorative and stressed behaviors.

The total variances explained by PC axes were much lower in MIS compared to OFT (54.0% of the total variance was covered by PC1 to PC3 in urban squirrels and 57.0% in rural squirrels) (Table 2, 3). PC1 of urban and rural squirrels included “T gaze”, “T stretch”, “T sniff”, “T touch”, and “T front”: therefore, I referred to it as "attention to conspecifics". PC2 of urban squirrels was correlated with agonistic behaviors such as “T

tail” and “N taps”, and other confounding behaviors: therefore, it is difficult to interpret. Since PC2 of rural squirrels was correlated with agonistic behaviors and vigilance behaviors, PC2 of rural squirrel could be characterized as aggressiveness. Other PC axes could not be clearly interpreted.

Most sets of behaviors in OFT (i.e. PCA axes) were repeatable (PC1 of urban OFT: repeatability = 66%, PC2: repeatability = 56%, PC3: repeatability = 53% PC1 of rural OFT: repeatability = 42%, PC2: repeatability = 52%, Table 2,3), while no repeatability was detected in PC3 of rural squirrels (PC3: repeatability = 9%; Table3). Some axes of MIS also had consistent individual variations (PC1 of urban; repeatability = 39%; PC3: repeatability = 37%, PC1 of Rural; repeatability = 38%; PC3: repeatability = 49%, Table 2,3), but no repeatability was detected in PC2 (urban; repeatability = 10%. Rural; repeatability < 0.1%, Table 2,3). Most of the independent behaviors of OFT (eight behaviors) were also significantly repeatable (mean \pm SD = 48.7% \pm 25.16, Table 4), whereas most behaviors of MIS (six behaviors) were not (mean \pm SD = 15.2% \pm 14.07, Table4).

Urban-rural comparisons

Since PCA axes were little different between urban and rural areas and were often difficult

to interpret, I compared basic (i.e., not summarized) behaviors between urban and rural squirrels. I selected all the behaviors from OFT and six from MIS that were obviously reacted to the mirror (i.e., T stretch, T sniff, T touch, T front, T tail, and N taps).

Contrary to the predictions, I found no significant differences in all behaviors between urban and rural squirrels (Figure 1,2. Table 5). In addition, no significant effect was detected in most of the fixed variables (season, sex, and its interaction; Table 5): only the N lines was significantly influenced by season, the interaction of season and areas, and the interaction of sex and season (Table 5).

Contrary to OFT and MIS, VED and the number of mobbing calls were significantly different between urban and rural habitats (VED, Estimate = -0.80, $SE = 0.27$, $t = -3.01$, $P < 0.001$; number of mobbing call, Estimate = 0.81, $SE = 0.24$, $t = 3.35$, $P < 0.001$; Table 7. Urban squirrels showed shorter VED compared to rural squirrels (mean $\pm SE$ of urban VED = $2.66 \pm 0.13m$, mean $\pm SE$ of rural VED = $5.42 \pm 0.35m$, Figure 3). The interaction of sex and season was also significant (Table 6). VED of spring male was longest and decreased in autumn (spring male; mean $\pm SE = 5.41 \pm 0.78m$, autumn male; mean $\pm SE = 3.65 \pm 0.28m$), while that of spring female was same degree of autumn and lower than VED of spring male (spring female; mean $\pm SE = 3.28 \pm 0.28m$, autumn female; mean $\pm SE = 3.19 \pm 0.27m$). In addition, I observed anti-predator

behaviors in 237 urban individuals for three years field sampling, and 34 individuals (14.3%) showed mobbing calls towards human. On the other hand, only 4 of 128 rural individuals (3.1%) showed mobbing calls. The mean number of mobbing calls was almost three times more in urban squirrels compared to rural one (Figure 3). The hurdle model confirmed that the mobbing behavior was significantly different between urban and rural individuals (Table 7). The interaction of sex and season was also significant, while other variables were not (Table 7).

Discussion

Contrary to the general predictions of urban animals shifting behaviors, I found no major differences in most of the behaviors examined between urban and rural squirrels, except for VED and the number of mobbing calls toward humans. Although OFT and MIS are traditional methods assessing animal behavior and our data suggested repeatability within individuals, no significant difference was observed between urban and rural individuals and no clear interpretable PC axes was detected even though each behavior seem to have biological meanings (Table 1). Notably, aggressive responses to conspecifics, which were measured in MIS, were not significantly different between two habitat types, but the intensity of mobbing calls toward humans was highly significant. Considering that the

remarkable difference of VED was observed toward humans, urban squirrels may respond only to direct human-related events. This suggests that urban animals could flexibly modify behaviors when necessary, while their basic characteristics (e.g., personality or temperament) remain unchanged. This also indicates that results can include significant biases if I assess the behavioral shift of urban animals only with the behaviors that are strongly tied to human presence, including FID and aggressiveness to human.

Usefulness of OFT and MIS

OFT and MIS have been widely used for many taxa, including mammals, birds, reptiles, and fishes, to assess behavioral characteristics, such as activity, boldness, exploration, aggressiveness, and sociability (e.g., Hall & Ballachey 1932; Boon et al. 2007; Martin & Real 2008). While these methods have been succeeded to a large extent, their PCA approaches often receive criticisms mainly derived from difficulties in understanding what characters were extracted from different behaviors (Carter et al. 2013; Perals et al. 2017). In North American red squirrels *Tamiasciurus hadsonicus* each PCA axis clearly reflected activity, aggressiveness, and exploration, respectively (Boon et al. 2007). This species is highly territorial and aggressive interactions are strong within populations (Smith 1968). Eurasian red squirrels, on the other hand, have no or weak territoriality,

and aggressive interactions are not usually observed (Wauters & Dhondt 1989; 1992), which might result in ambiguous behavioral characteristics. The usefulness of PCA approaches in OFT and MIS may depend on species and further empirical studies are needed.

Does urbanization affect behavior/personality?

Differences of VED and the number of mobbing call to human were highly significant between urban and rural squirrels. This is consistent with previous studies suggesting that urban animals become bolder and more aggressive (Evans et al. 2010; Lowry et al. 2013). However, no significant difference was observed in any of the 15 behavioral variables measured in OFT and MIS. This was surprising considering the remarkable difference in VED (and other flight distances, Uchida et al. 2016), the number of mobbing calls, and also many past studies supporting behavioral shifts (e.g., Miranda et al. 2013; Atwell et al. 2012; Carrete & Tella 2017). We can interpret that urban animals may change behavior only toward human approaches and the essential characters might not. Since most previous studies did not employ flight distances and OFT/MIS simultaneously, responses to human-specific events might have been overlooked.

Only a few studies used OFT/MIS investigating the behavioral difference

between urban and rural mammals (Martin & Réale 2008; Lyons et al. 2017; Hurtado & Mabry 2017). Lyons et al. (2017) demonstrated in Eastern chipmunks *Tamias striatus* that habitat type (urban vs. natural) significantly affects a set of behavioral variables (multivariate model). However, while the authors claimed that locomotion, grooming, and latency contribute to the difference, none of the eight behavioral variables measured was significantly different between urban and natural habitat (univariate model). Therefore, some behavioral shift would have occurred in the chipmunk, urban-natural difference is not necessarily clear. In addition, Hurtado & Mabry (2017) used MIS to assess aggressiveness and boldness in Kangaroo rat *Dipodomys merriami* but found no significant difference between urban and natural habitats. More research is clearly needed to assess the general patterns of behavioral changes in mammals living in urban environments.

Some researchers have attempted to link animal personality to urban adaptation (Scales et al. 2011; Miranda et al. 2013). This implies that behavioral shifts in urban individuals and behavioral consistency within individuals are partially due to genetic changes (Carrete et al. 2016). I found that individual behaviors were mostly consistent across time with the maximum of one year, but clear personality traits which are suggested in previous studies (e.g. exploration, sociability, aggressiveness) were not

extracted from PCA analyses. While each individual squirrel would have some behavioral tendency, it remains unknown whether the personality approach is useful for this species. No differences in basic behaviors but significant differences in human-related behaviors between urban and rural habitats suggested that squirrels can plastically change their behaviors depending on the context and situation rather than microevolution.

Given the apparent differences between urban and rural habitats, why no difference was observed in basic behaviors? There may be many confounding factors. First, environments might be rather similar for squirrels. While there are many artificial structures in urban parks, forest patches generally remain in some areas within parks. Such forests may be similar to natural habitats. However, this is not likely because the squirrels use non-forest areas such as the managed lawns of playgrounds, and sometimes cross the roads to move to the next park or garden. In particular, they went to almost any area if artificial feeding was provided (K. Uchida, personal observation). Second, different selection pressures appear in urban habitats but the effects are similar to those in natural habitats. Increased boldness is generally considered as a result of predator release (Møller 2008). In fact, natural predators, such as foxes and raptors, were much rare in urban parks compared to rural forests (K. Uchida, personal observation). In addition, in the Obihiro city where winter cold is very harsh, novel meso-predators, such

as domestic cats, are also uncommon (K. Uchida, personal observation). However, road kill is frequently observed for the squirrels, and thus losing vigilance seems to be maladaptive. The major stressor in natural habitats should be predation, whereas in urban habitats it may be some artificial structures: these are certainly different, but the level of stress might be similar. Third, selection pressures may change throughout time and the advantages of increased boldness, exploration, and aggressiveness may become no longer significant. For example, exploration to find new resources and habitats should be adaptive in the initial stage of population establishment in urban areas (Dingemanse et al. 2013), but the efficient use of local resources should be more adaptive after the population reaches or is near carrying capacity (Sol et al. 2013; Lyons et al. 2017). In summary, agents affecting animal behavior are various and confounding each other, especially in urban environments. Responses to different habitats are also highly species specific. Therefore, I cannot draw strong conclusions about how urbanization affects animal behaviors until more studies have been conducted. This study underscores the importance of assessing basic behaviors and human-induced behaviors simultaneously.

Figure Legends

Figure 1

Time proportion of behaviors extracted from OFT and MIS in urban and rural Eurasian red squirrels. Refer Table 1 for each behavior. Plots show medians, quartiles, and ranges. “U” and “R” represent urban and rural areas, respectively.

Figure 2

Count data for behaviors extracted from OFT and MIS in urban and rural Eurasian red squirrels. Refer Table 1 for each behavior. Plots show medians, quartiles, and ranges. “U” and “R” represent urban and rural areas, respectively.

Figure 3

VED (a) and the number of mobbing calls (b) in urban and rural Eurasian red squirrels.

Tables

Table 1.

Details for the behaviors of Eurasian red squirrels observed in OFT and MIS. Sources of definition of each behavior are also shown. I used our original definitions for some behaviors such as N taps because such behaviors were not described in previous studies. Each behavior can be tentatively categorized into some behavioral types or personality traits based on previous studies.

Behaviors	Definitions	Presumed behavioral categories	Sources
OFT			
N lines	number of lines crossed	Activity	Petelle& Blumstein 2014
T locomotion	proportion of time working in arena	Activity	Boon et al. 2007

T still	proportion of time sitting motionless	Activity	Boon et al. 2008
N jumps	number of jumps	Activity	Petelle& Blumstein 2014
T stand	proportion of time standing on hind legs and scanning	Activity/Exploration	Boon et al. 2008; Petelle& Blumstein 2014
T scan	proportion of time waving head and scanning	Exploration	K.Uchida personal observation
N head dips	number of head dips into hole in floor	Exploration	Boon et al. 2008
T groom	proportion of time self-grooming	Stress response	Boon et al. 2008
Pellet	whether pellets were left in the arena or not	Stress response	Boon et al. 2008

MIS

T locomotion	same as OFT	Activity	
T still	same as OFT	Activity	
T groom	same as OFT	Stress response	

T stand	same as OFT	Activity/Exploration	
T scan	same as OFT	Exploration	
T gaze	proportion of time gazing at the mirror	Sociability	K.Uchida personal observation
T stretch	proportion of time slowly approach towards mirror with back legs stretched behind	Sociability	Boon et al. 2008
T sniff	proportion of time sniff the mirror	Sociability	Petelle& Blumstein 2014
T touch	proportion of time touch and look into the mirror	Sociability	K.Uchida personal observation
T front	proportion of time staying within the third of arena closest to the mirror	Sociability	Petelle& Blumstein 2014
T tail	proportion of time raising the tail	Aggressiveness	Boon et al. 2008
N taps	number of taps on the floor	Aggressiveness	K.Uchida personal observation

Table 2. The results of PCA and repeatability test for OFT and MIS in urban Eurasian red squirrels. The behaviors that contributed importantly to each component are shown with bold type. Detail of each behavior is described in Table 1.

OFT	PC1	PC2	PC3	MIS	PC1	PC2	PC3
N line	-0.42	0.09	-0.17	T locomotion	-0.26	-0.46	-0.06
T locomotion	-0.45	0.14	-0.13	T still	0.12	0.11	-0.78
T still	0.29	0.57	-0.03	T groom	-0.28	-0.03	0.12
N jumps	-0.38	0.17	-0.34	T stand	-0.30	-0.23	0.17
T stand	-0.24	-0.47	0.38	T scan	-0.27	0.32	0.23
T scan	0.33	-0.39	-0.26	T gaze	0.36	-0.13	0.51
N head dips	-0.41	0.11	-0.02	T stretch	-0.23	-0.29	0.05

T groom	0.02	0.43	0.67	T sniff	-0.36	0.10	0.00
Pellet	-0.22	-0.24	0.43	T touch	-0.44	0.21	-0.03
				T front	-0.40	0.09	-0.07
				T tail	0.00	-0.51	-0.14
				N taps	-0.05	-0.45	-0.10
SD	2.12	1.11	1.08		1.85	1.26	1.23
Total variance	0.50	0.14	0.13		0.28	0.13	0.13
Repeatability	0.66	0.56	0.53		0.39	0.10	0.37
95%CI	0.45-0.82	0.32-0.77	0.26-0.76		0.12-0.63	0-0.42	0.03-0.63
LRT (d.f.=1)	9.11	15.90	13.00		12.10	0.37	1.71
<i>p</i> -value	<0.001	<0.001	<0.001		<0.001	0.27	0.02

Table 3. The results of PCA and repeatability test for OFT and MIS in rural Eurasian red squirrels. The behaviors that contributed importantly to each component are shown with bold type. Detail of each behavior is described in Table 1.

OFT	PC1	PC2	PC3	MIS	PC1	PC2	PC3
N line	-0.46	-0.05	-0.20	T locomotion	-0.49	0.27	-0.10
T locomotion	-0.50	-0.02	-0.26	T still	0.05	-0.12	0.40
T still	0.24	-0.50	0.20	T groom	-0.14	0.19	-0.23
N jumps	-0.37	0.08	-0.35	T stand	-0.12	0.21	0.35
T stand	-0.33	0.30	0.47	T scan	0.04	-0.04	0.52
T scan	0.40	0.22	-0.42	T gaze	0.36	0.03	-0.59
N head dips	-0.22	-0.56	-0.12	T stretch	-0.39	0.23	-0.09

T groom	0.00	-0.54	0.02	T sniff	-0.35	-0.48	-0.12
Pellet	-0.17	0.01	0.56	T touch	-0.30	-0.50	-0.07
				T front	-0.22	-0.41	-0.03
				T tail	-0.25	0.09	-0.13
				N taps	-0.34	0.34	0.00
SD	1.83	1.18	1.14		1.74	1.50	1.24
Total variance	0.37	0.16	0.15		0.25	0.19	0.13
Repeatability	0.42	0.52	0.09		0.38	< 0.001	0.49
95%CI	0-0.73	0.12-0.79	0-0.51		0-0.72	0-0.496	0.071-0.79
LRT (d.f.=1)	4.73	3.96	0.01		5.19	0.00	8.81
<i>p</i> -value	0.02	0.02	0.46		0.01	1.00	<0.001

Table 4. The result of repeatability test for each behavior in urban and rural Eurasian red squirrels measured by OFT and MIS.

		Repeatability	LRT (d.f.=1)	95%CI	<i>p</i> -value
OFT					
	urban	0.35	0.40	0.05-0.64	0.26
N line	rural	0.24	1.08	0-0.703	0.15
	urban	0.65	10.50	0.42-0.81	<0.001
T locomotion	rural	0.34	2.77	0.001-0.76	0.00
	urban	0.48	12.60	0.21-0.72	<0.001
T stand	rural	0.81	14.90	0.63-0.94	<0.001
	urban	0.48	13.90	0.24-0.73	<0.001
T still	rural	0.49	3.20	0.04-0.82	0.04
	urban	0.58	8.90	0.33-0.78	0.00
T scan	rural	0.55	8.99	0.19-0.56	0.00
	urban	0.63	13.10	0.53-0.85	<0.001
T groom	rural	<0.001	<0.001	<0.001-<0.001	0.50
N head dips	urban	0.89	35.70	0.81-0.94	<0.001

	rural	0.65	9.29	0.36-0.89	0.00
	urban	0.64	5.72	0.42-0.81	0.01
N jumps	rural	<0.001	<0.001	<0.001-<0.001	0.50
MIS					
	urban	0.22	1.70	0-0.57	0.10
T stretch	rural	0.37	4.60	0-0.76	0.00
	urban	0.00	0.00	0-0.3	1.00
T sniff	rural	0.02	0.04	0-0.66	0.42
	urban	0.12	1.01	0-0.45	0.15
T touch	rural	0.12	0.09	0-0.66	0.38
	urban	0.21	1.53	0-0.55	0.11
T front	rural	0.12	0.09	0-0.66	0.38
	urban	0.45	8.60	0.15-0.68	0.00
T tail	rural	0.12	1.12	0-0.76	0.15
	urban	0.08	0.20	0-0.42	0.32
N taps	rural	0.00	<0.001	0-0.65	1.00

Table 5. The results of generalized linear mixed models of OFT and MIS in Eurasian red squirrels. Bold letters reflect statistically significant variables. Measurements for time proportion and count measurements were divided into column.

T locomotion	Estimate	SE	df	t value	P	N lines	Estimate	SE	z value	P
(Intercept)	0.14	0.07	42.04	2.12	0.04	(Intercept)	3.42	0.27	12.84	<0.001
AREA (rural vs urban)	0.08	0.08	40.56	0.98	0.33	AREA (rural vs urban)	0.27	0.30	0.88	0.38
SEX (female vs male)	0.11	0.08	43.92	1.28	0.21	SEX (female vs male)	0.46	0.32	1.40	0.16
SEASON (autumn vs spring)	-0.07	0.07	82.18	-1.03	0.31	SEASON (autumn vs spring)	-0.35	0.10	-3.34	<0.001
AREA × SEX	-0.07	0.10	40.80	-0.69	0.50	AREA × SEX	-0.46	0.38	-1.19	0.23
AREA × SEASON	0.07	0.07	84.78	1.08	0.28	AREA × SEASON	0.44	0.10	4.40	<0.001

SEX × SEASON	0.04	0.07	84.43	0.54	0.59	SEX × SEASON	0.23	0.10	2.38	0.02	
<hr/>						<hr/>					
T groom						N jumps					
<hr/>						<hr/>					
(Intercept)	0.04	0.02	49.83	2.31	0.03	(Intercept)	7.479	6.462	1.157	0.254	
AREA (rural vs urban)	-0.01	0.02	48.44	-0.68	0.50	AREA (rural vs urban)	4.078	7.327	0.557	0.581	
SEX (female vs male)	0.00	0.02	51.75	0.09	0.93	SEX (female vs male)	9.014	8.037	1.122	0.269	
SEASON (autumn vs spring)	-0.01	0.02	83.29	-0.56	0.58	SEASON (autumn vs spring)	-10.368	7.096	-1.461	0.148	
AREA × SEX	0.01	0.02	48.70	0.21	0.83	AREA × SEX	-1.175	9.267	-0.127	0.9	
AREA × SEASON	0.01	0.02	85.34	0.82	0.42	AREA × SEASON	10.983	6.963	1.577	0.118	
SEX × SEASON	-0.01	0.02	85.20	-0.42	0.68	SEX × SEASON	0.497	6.878	0.072	0.943	
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T stand						N head dips					
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(Intercept)	0.27	0.06	52.56	4.68	<0.001	(Intercept)	1.62	0.34	4.81	<0.001	

AREA (rural vs urban)	-0.08	0.07	50.94	-1.26	0.22	AREA (rural vs urban)	0.46	0.38	1.22	0.22
SEX (female vs male)	-0.12	0.07	54.54	-1.69	0.10	SEX (female vs male)	0.06	0.41	0.15	0.88
SEASON (autumn vs spring)	-0.06	0.06	90.59	-0.95	0.34	SEASON (autumn vs spring)	0.28	0.23	1.20	0.23
AREA × SEX	0.05	0.08	51.19	0.64	0.52	AREA × SEX	-0.01	0.48	-0.02	0.98
AREA × SEASON	0.05	0.06	92.70	0.90	0.37	AREA × SEASON	-0.09	0.23	-0.39	0.69
SEX × SEASON	0.11	0.06	92.39	1.93	0.06	SEX × SEASON	0.14	0.22	0.65	0.52
<hr/>						<hr/>				
T tail						N taps				
<hr/>						<hr/>				
(Intercept)	139.70	46.76	51.04	2.99	<0.001	(Intercept)	12.68	6.97	1.82	0.08
AREA (rural vs urban)	-23.32	52.93	49.02	-0.44	0.66	AREA (rural vs urban)	-10.74	7.78	-1.38	0.18
SEX (female vs male)	42.59	58.25	52.51	0.73	0.47	SEX (female vs male)	9.48	8.64	1.10	0.28
SEASON (autumn vs spring)	-52.97	55.00	97.45	-0.96	0.34	SEASON (autumn vs spring)	-6.53	10.92	-0.60	0.55

AREA × SEX	-29.28	66.94	48.91	-0.44	0.66	AREA × SEX	-10.78	9.75	-1.11	0.28
AREA × SEASON	88.61	53.89	99.63	1.64	0.10	AREA × SEASON	3.56	10.63	0.33	0.74
SEX × SEASON	-20.59	53.25	99.07	-0.39	0.70	SEX × SEASON	6.86	10.53	0.65	0.52

T sniff

(Intercept)	0.01	0.01	65.05	0.55	0.58
AREA (rural vs urban)	0.00	0.01	60.85	0.29	0.77
SEX (female vs male)	0.00	0.01	61.75	-0.19	0.85
SEASON (autumn vs spring)	-0.02	0.02	110.42	-1.00	0.32
AREA × SEX	-0.01	0.01	57.59	-0.40	0.69
AREA × SEASON	0.02	0.01	110.96	1.50	0.14
SEX × SEASON	0.03	0.01	110.70	2.11	0.04

T front

(Intercept)	0.13	0.10	52.62	1.21	0.23
AREA (rural vs urban)	0.09	0.12	49.81	0.79	0.43
SEX (female vs male)	-0.06	0.13	52.21	-0.44	0.67
SEASON (autumn vs spring)	0.18	0.14	105.25	1.32	0.19
AREA × SEX	-0.07	0.15	48.43	-0.49	0.62
AREA × SEASON	-0.08	0.14	106.84	-0.62	0.54
SEX × SEASON	0.01	0.13	106.25	0.06	0.96

T look with touch mirror

(Intercept)	0.00	0.02	67.03	0.27	0.79
AREA (rural vs urban)	0.01	0.02	62.25	0.60	0.55

SEX (female vs male)	0.00	0.02	62.82	-0.09	0.93
SEASON (autumn vs spring)	0.02	0.03	111.02	0.76	0.45
AREA × SEX	-0.01	0.03	58.44	-0.58	0.56
AREA × SEASON	-0.02	0.03	111.39	-0.63	0.53
SEX × SEASON	0.03	0.03	111.19	0.96	0.34

Table 6. The results of generalized linear mixed models of VED in Eurasian red squirrels.

Bold letters reflect statistically significant variables. Measurements for time proportion and count measurements were divided into column.

Variables	Estimate	SE	d.f.	t value	P
(Intercept)	0.37	0.23	58.79	1.62	0.11
AREA (rural vs urban)	-0.80	0.27	55.83	-3.01	<0.001
SEX (female vs male)	0.13	0.29	44.51	0.45	0.66
SEASON (autumn vs spring)	0.42	0.26	147.45	1.63	0.11
AREA × SEX	-0.25	0.34	40.80	-0.74	0.47
AREA × SEASON	-0.52	0.27	154.98	-1.92	0.06
SEX × SEASON	0.62	0.27	156.95	2.28	0.02

Table 7. The result of the hurdle model for the number of mobbing calls in Eurasian red squirrels. Bold letters reflect statistically significant variables.

	Estimate	SE	t value	P
Rate of mobbing (Zero model)				
(Intercept)	-3.62	1.04	-3.48	<0.001
AREA (rural vs urban)	1.81	1.08	1.67	0.09
SEX (female vs male)	-2.03	1.42	-1.43	0.15
SEASON (autumn vs spring)	0.64	1.30	0.50	0.62
AREA × SEX	1.99	1.38	1.44	0.15
AREA × SEASON	-1.51	1.37	-1.10	0.27
SEX × SEASON	1.46	0.79	1.85	0.06
Number of mobbing (Count model)				
(Intercept)	2.89	0.24	12.26	<0.001
AREA (rural vs urban)	0.81	0.24	3.35	<0.001
SEX (female vs male)	-0.37	0.50	-0.74	0.46
SEASON (autumn vs spring)	0.00	0.33	0.00	1.00
AREA × SEX	0.19	0.49	0.39	0.70
AREA × SEASON	-0.24	0.35	-0.69	0.49
SEX × SEASON	-0.73	0.16	-4.67	<0.001

Fig.1

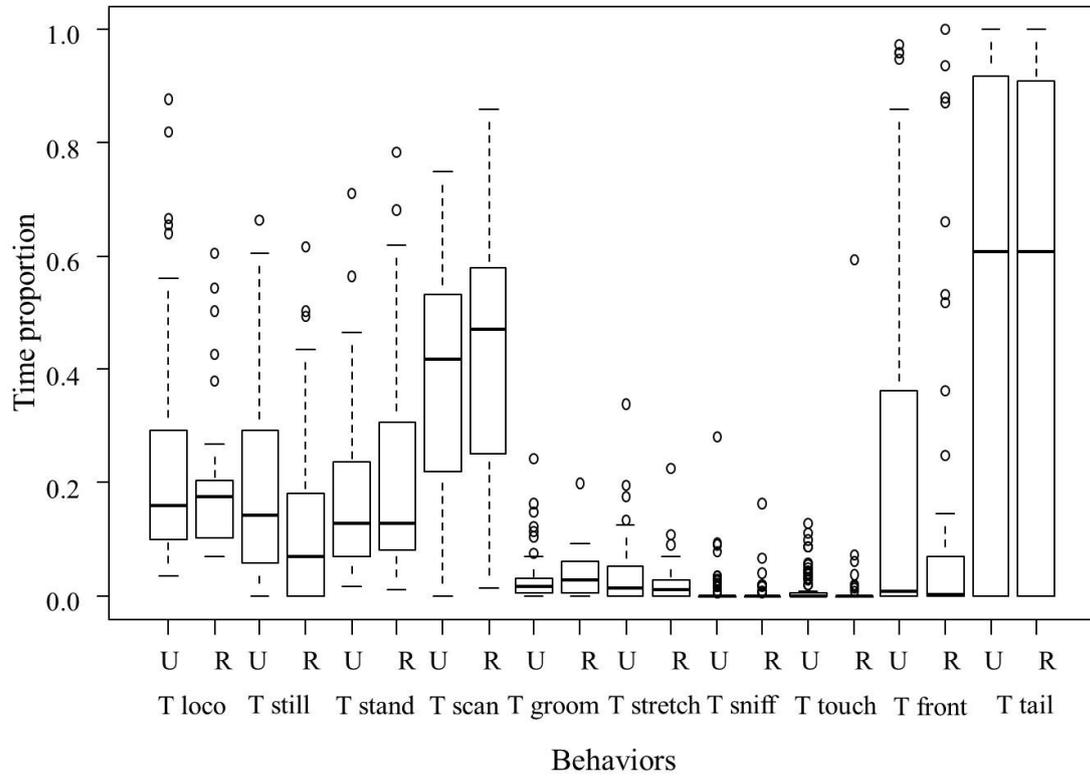


Fig.2

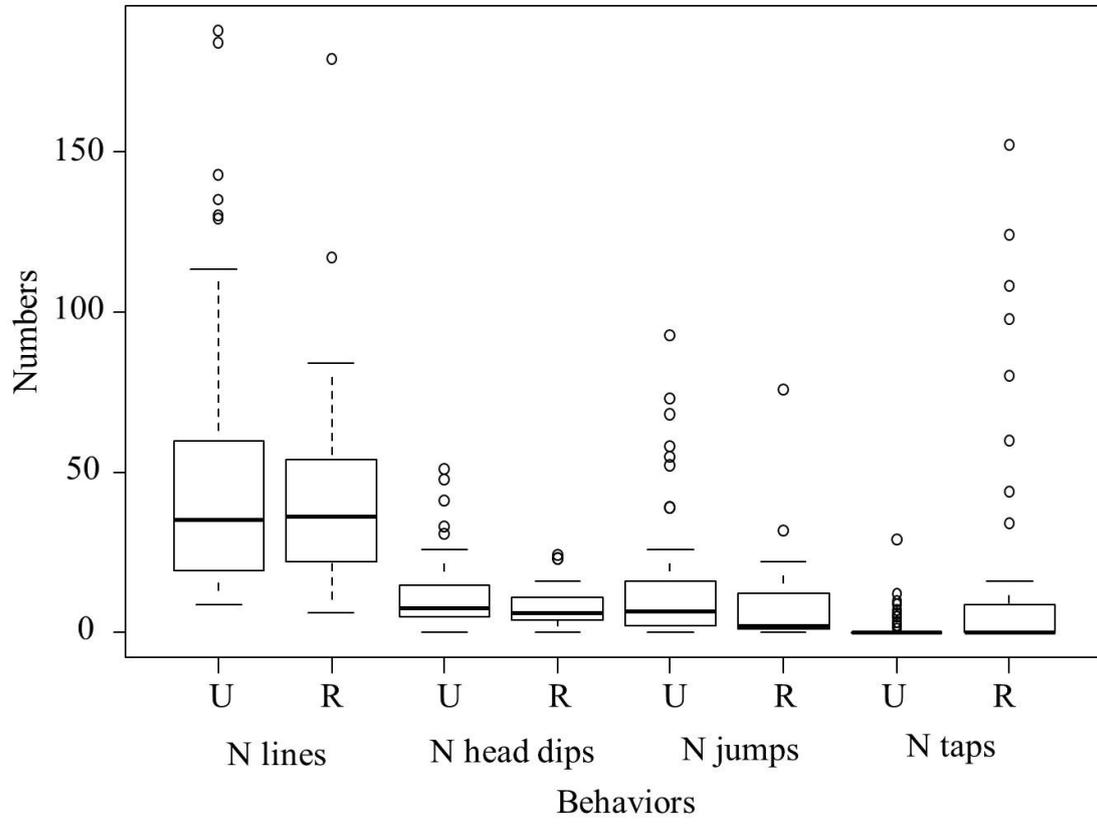
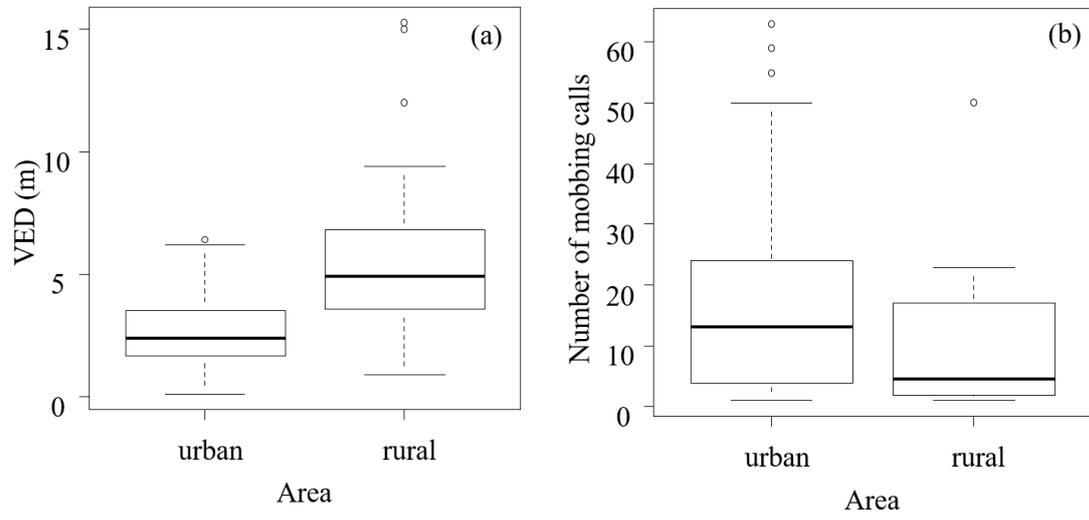


Fig.3



CHAPTER 4

Urban squirrels can assess risk levels of different potential predators



Abstract

Increased boldness is one of the most prevalent behavioral modifications in urban animals to cope with anthropogenic environmental alterations. Most previous studies showed enhanced boldness by some responses to human approach, such as the reduction of flight initiation distance (FID). However, this includes two confounding factors related to “boldness”, i.e., reduction of vigilance and habituation to human. Ignoring the totally different processes will mislead our understanding of urban adaptation and the proper management of urban wildlife. Here, I propose a simple framework to separate the two processes using two flight distances toward different approaching threats. I considered that the distance at which focal individuals notice an approaching object (i.e. alert distance, AD) represent more on vigilance, whereas FID represent on risk assessment, which is related to habituation. I applied a predictive framework to Eurasian red squirrels using AD and FID to multiple threats with different risk levels (i.e. human, predator, and novel object). I found that while urban individuals reduced vigilance they could still assess different risk levels. The reduction of FID to human was largely (59-67%) due to habituation rather than reduction of vigilance. Unexpectedly, urban squirrels reacted more strongly to the novel object compared to rural conspecifics, indicating neophobic tendency. Our framework can be easily applied to many animals and significantly

contribute to the understanding of urban adaptation.

Introduction

The rapid increase of urbanization has led to dramatic alterations of natural habitats over the last centuries (Gaston 2010). In order to cope with such disturbances, some animals have modified their behaviors and life histories, such as foraging mode, home range size, temporal activity patterns, migration tendency, and personality traits (Shochat et al. 2006; Tuomainen & Candolin 2011; Lowry et al. 2013; Gaynor et al. 2018). Increased boldness is one of the most widely reported behavioral shifts in many urban animals (Møller 2008, 2012; Tuomainen & Candolin 2011; Lowry et al. 2013; Díaz et al. 2013). Since large predators avoid anthropogenic environments (Bateman & Fleming 2012), predation risk in urban areas tends to decline (Møller 2012). As a result, prey animals become less vigilant, which would be adaptive response to urbanization (Lowry et al. 2013).

Increase of boldness in urban environment is most frequently assessed by flight initiation distance (FID), the distance at which individuals start to flee from an approaching object (Short & Petren 2008; Møller 2010; Atwell et al. 2012; Uchida et al. 2016). FID has well-developed theoretical bases on optimal escaping theory (Ydenberg

& Dill 1986; Blumstein 2006; Cooper & Frederick 2007, Uchida et al. 2016) and is easily applied to many species: researchers approach to target animals and measure the distance. However, while FID should partly reflect the reduction of vigilance, another confounding factor exists, i.e., habituation to human. Literature on animal behavior or animal personality often uses the term “boldness”, but rarely define the terminology. Here, I considered that boldness consists of two components, i.e., boldness to general threats, which should be related to vigilance, and boldness to specific threats, which should be related to habituation or risk assessment to specific targets. Ignoring the totally different components will significantly bias the interpretation of the results. For example, the reduction of FID to human approach does not necessarily mean that the urban animals lost vigilance or anti-predator response and, hence, become susceptible to novel meso-predators in urban environments such as ; cats (Geffroy et al. 2015). Surprisingly, very few studies have attempted to measure FID to non-human objects to understand how urban animals can assess different risk levels (but see, McCleery 2009, and Rodriguez-Prieto et al. 2009).

Here, I propose a novel framework discriminating the effects of reduction of vigilance and habituation to human on the increased boldness of urban animals. This will be accomplished by comparing two commonly used flight distances between urban and

rural (natural) habitats toward multiple threats with different risk levels (Fig. 1). Alert distance (AD) is the distance at which a target animal notices an approaching object and, therefore, should reflect a basic vigilance level (Fernández-Juricic et al. 2003; Bedoya-Perez et al. 2013; Cooper & Blumstein 2014). Animals cannot identify what is the approaching object and how risky it is until they identify the object (e.g. via eye sight, smell, or sound). Therefore, AD should not differ among approaching objects. 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 & Blumstein 2005; Samia et al. 2015; Cooper & Blumstein 2014). Accordingly, we would be able to assess the reduction of vigilance with AD and habituation to specific objects with FID (Fig. 1). I considered it best to use human, predator (model), and novel object (control) as approaching objects: FID should be longest for predator in natural condition, and FID will be reduced specifically to human in urban environment if habituation occurs. There should be three alternative scenarios for the increase of boldness in urban animals (Fig. 1). Scenario I: Increased boldness is entirely due to reduced vigilance. In this case, AD and FID will be reduced in urban compared to rural habitats, whereas the shape of FID will not change (e.g. longest for predator). 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 to human approach. Scenario III: Increased boldness is due to both processes. In this case, AD and FID will be reduced in urban habitat, and the shape of FID will also change.

I applied this framework to Eurasian red squirrels *Sciurus vulgaris* in central Hokkaido, Japan. Squirrels are good models to understand the adaptation to urban environments because many species have well adjusted to cities areas worldwide (Bateman & Fleming 2013; Fey & Selonen 2016; Uchida et al. 2016; Jokimäki et al. 2017). In addition, habitation to human is strongly expected because many squirrels in urban environments rely on artificial feeding (Uchida et al. 2016; Jokimäki et al. 2017).

METHODS

Study area and species

This study was carried out in the Tokachi region, central Hokkaido, Japan during 5th -27th October 2014. Obihiro is the largest city (the population size is about 160,000 people) located in the center of the Tokachi plain, which is surrounded by the rural agricultural land. I selected six city parks as urban sites and four forests as rural sites. All sites were isolated (at least 1 km apart from the nearest habitat patches) and individuals rarely move

among sites especially in such a short period (< three weeks). The urban and rural areas differed in human density, artificial structures (e.g. houses, buildings, roads) and traffic levels. Urban habitats generally contain recreational playgrounds or pathways and are surrounded by dense residential areas and wide roads. On the other and, rural habitats are surrounded by agricultural lands or next to the mountains. While some forests have man-made structures, I chose the sites without such structures. Vegetation, especially underbrush, in urban parks was cut lower than the fifty centimeters by park managers. During our field period, vegetation of rural sites was withered and, therefore, the visibility was not different between urban and rural sites. There is year-round artificial feeding from citizen in all of the urban sites. I rarely encountered human and never observed artificial feeding at the rural sites at least for four years since I started the research on squirrels in these populations. Red fox (*Vulpes vulpes*) and raptors, such as Eurasian hobb (*Falco Subbuteo*), Goshawk (*Accipiter gentiils*), and Sparrowhawk (*A. nisus*), are the main predators of red squirrels in Hokkaido, similar with European countries (Selonen et al. 2010). These predators were much less common in urban areas compared to rural areas (K. Uchida, personal observation). In addition, domestic cats, potential novel predator in urban area (Jokimäki et al. 2017), were remarkably rare in urban areas. Domestic dogs were obligated to be lead and I

saw no feral dogs in urban areas.

Field survey

Field survey was carried out from sunrise (typically between 0430 and 0500) to 10:00 am, which is the most active time for squirrels (Ikeda et al. 2016). To reduce the effects of observer's bias on AD and FID, data collection was conducted by the first author (K. Uchida). I randomized the sites to visit each day. In each site, I targeted only individuals foraging on the ground. I identified individuals using their characteristics (e.g. body size, condition, and coat color) to avoid resampling. Each squirrel was assigned to one of the following treatments: (1) human (the observer), (2) stuffed red fox as a predator, and (3) stuffed red fox covered with a black plastic bag as a control (or novel object). The stuffed fox and the novel object were fixed on a cart, which was attached to a long steel pole (6 m). When moving the objects (cart) from the other side of the pole, the observer was hidden behind an opaque board (95cm × 65cm): squirrels paid more attention to the objects than the board. Each object was approached to squirrels at a constant speed of 1.0 m/s (i.e. human walking speed). Since the start distance potentially influences the flight distances (Rodriguez-Prieto *et al.*, 2009), I recorded the data only when the start distance was 40 m or longer, which was considered as far enough for squirrels not to notice an

approaching object. When a target squirrel raised its head and looked towards one of the approaching objects, the distance between the object and the squirrel was measured as AD (Uchida et al. 2017). I continued to approach to the squirrel until it fled: then, the distance was measured as FID (Uchida et al. 2017). I marked the points of AD and FID by dropping tags while approaching to a target squirrel and measured after the trial. The distances were measured by a laser rangefinder (tru-Pulse 200, Laser technology Inc., Centennial, Colorado, U.S.). Observation was not carried out on rainy, foggy and windy days in order to avoid the potential difference of squirrels' detectability to threats. Data collection was also terminated when one of followings occurred: (1) when the targeted squirrels responded to alarm call by conspecifics or avian species (e.g. Eurasian jays, *Garrulus glandarius*), (2) when pedestrians/bicycles approached towards the focal squirrels, and (3) when the targeted squirrels approached to the observer.

Data analyses

I ran two-way ANOVAs to examine the effects of areas (urban vs rural), objects (human, predator, and novel object) and their interaction on 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 area, $F = 1.294, p = 0.280$; AD and object, $F = 4.031, p = 0.048$; FID and area, $F = 1.345, p = 0.264$; FID and object, $F = 1.617, p = 0.206$). When ANOVA was significant ($P < 0.05$), I performed a posthoc test using Turkey's multiple comparison. All statistical analyses were carried out using R software, Version 3.0.1 (R Development Core Team, 2015).

Results

I recorded 81 AD (urban area: human $N = 17$, predator $N = 18$, novel object $N = 11$, rural area: human $N = 12$, predator $N = 14$, novel object $N = 9$) and 137 FID (urban area: human $N = 24$, predator $N = 29$, novel object $N = 25$, rural area: human $N = 25$, predator $N = 19$, novel object $N = 15$). AD was significantly shorter in urban area compared to rural one (mean \pm S.E.: 19.9 ± 1.0 m for urban area, 27.6 ± 1.6 m for rural area), whereas no significant difference was observed among different objects (two-way ANOVA; Fig. 2, Table 1). For FID not only the area, but also the object and interaction effects were detected (Fig. 2, Table 1). Mean FID of urban squirrels was just half of the FID of the rural counterpart (mean \pm S.E.: 9.6 ± 0.8 m for urban area, 19.1 ± 1.1 m for rural area). Turkey's multiple comparisons showed that FID of urban squirrels towards human and predator were significantly reduced compared to rural squirrels (Fig. 2, Table 2), whereas

FID to the novel object was not statistically different. In addition, FID towards human was significantly shorter than the fox model and novel object in urban squirrels but no significant difference was observed among the objects in rural area (Fig. 2, Table 2). In short, the response to human was the most remarkably modulated in urban area (mean \pm S.E.: 4.5 ± 0.7 m for urban area, 18.5 ± 1.7 m for rural area) and FID was reduced also toward the predator model (mean \pm S.E.: 11.1 ± 1.0 m for urban area, 21.0 ± 1.8 m for rural area). Overall, the results were most consistent with the Scenario III (Fig. 1 and 2), although some deviations from the predictions were observed (i.e. reduction of FID to predator and no reduction of FID to novel object).

Discussion

While it has been widely reported that urbanization increases the boldness of wide range of animals (e.g. Møller 2008; Evans et al. 2010; Samia et al. 2017), few studies have clarified the underlying processes. I proposed a simple framework to separate the effects of reduction of vigilance and habituation to human on increased boldness by comparing AD and FID to multiple objects between urban and rural habitats. I applied this to Eurasian red squirrels and found that both reduction of vigilance and habituation contribute to the increased boldness in urban habitats. Since AD and FID are widely used

in many animals (Stankowich & Blumstein 2005), this novel framework would contribute to the understanding of urban adaptation, as well as the effective management of urban animals.

Bold urban squirrels can assess different risk levels

Our conceptual model assumes the reduction of vigilance and habituation as the main drivers of increased boldness in urban habitats. The distance at which an animal notices an approaching object (AD) should reflect the vigilance and should not depend on the approaching object. As expected, AD did not depend on the object types, whereas it was significantly reduced in urban than rural habitats, indicating the reduction of vigilance.

Animals may notice a larger approaching object sooner than smaller ones. In our experiment, the predator model was much smaller (e.g. 0.5m in height) than human, but the AD did not differ among the objects. Squirrels might notice an approaching object first via sounds rather than visuals. Regardless of the squirrels' perception systems, AD should be a proper indicator to assess the vigilance. Reduction of vigilance in urban squirrels is probably due to the loss of natural predators, as suggested by other urban animals (Møller et al. 2008).

I also assumed that the distance at which an animal fled (FID) should depend on the approaching object and, therefore, reflect how familiar and risky the object is. The most remarkable result is the high tolerance of urban squirrels to human approach, strongly suggesting habituation. Although human is generally considered as a top predator and act as stressors for many animals (e.g. “super predator”; Darimont et al. 2015), human sometimes make affinity relationships with wild animals such as small bird and mammals: human feed animals and animals rely on such artificial feeding (Uchida et al. 2016; Jokimäki et al. 2017). In fact, during our field survey squirrels often approached to us and even climbed our body, probably begging for foods. Therefore, in order to better utilize resources induced by human, tolerance to human would be a big advantage in urban environments.

FID to the predator model was significantly decreased in urban habitat, while FID to the novel object did not differ between urban and rural habitats. This is somewhat unexpected because if urban animals increase boldness they should also show the reduction of FID to novel objects. Or, if urban animals still care about something (e.g. novel objects) they should also do so for predators. There are two explanations for the 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 tendency in urban animals (e.g. Miranda et al. 2013; Audet et al. 2015; Federspiel et al. 2016). Alternative explanation stems from visual category recognition: habituation to one stimulus can transfer to other stimuli when both stimuli are classified by a similar type (Blumstein 2016; Geffroy et al. 2016). In our study sites, human often take walks with dogs in urban parks and dogs usually do not care about squirrels. Since dogs and foxes are both medium sized canids, habituation to the domestic dogs might reduce the FID towards the fox model (McCleery 2009).

Surprisingly few studies have compared flight responses of urban and rural animals toward multiple objects (but see McCleery 2009, and Rodriguez-Prieto et al. 2009; Bateman & Fleming 2014). By doing so, I clearly demonstrated that even a small rodent could properly assess different risk levels: animals may possess higher cognitive abilities than I have initially thought. Moreover, some recent studies suggested that the cognition of urban individuals is enhanced in rapidly changing urban environment (Griffin et al. 2017). Because urban environments contain many novel stimulus induced by human activities, animals may require high behavioral flexibility to better utilize urban resources (Sol et al. 2002, 2013; Griffin et al. 2016). While Eurasian red squirrels around the Obihiro city decreased vigilance level, they can still assess the different risk levels. This

flexibility may be one of the reasons why squirrels have been succeeded to colonize to urban areas worldwide (Uchida et al. 2016; Jokimäki et al. 2017).

Usefulness of our framework

Past studies have shown the increased boldness in urban animals and attributed the causes either to the release from predators or habituation to human (Lowry et al. 2013). Since these mechanisms are totally different, it is necessary to separate these effects to better understand the adaptation to urban landscapes. Our approach enables to distinguish the alternative scenarios for the increase of boldness (Fig. 1). In addition, this also has a potential to quantitative assessments for the relative effects of decreased vigilance and habituation to human.

Consider that the FID consists of the components of decreased vigilance and habituation to human (Fig. 3). The former can be measured as the reduction of AD:

$$\text{Decreased vigilance by \%: } (1 - AD_u/AD_r) * 100$$

where the subscripts represent urban and rural, respectively.

The latter, on the other hand, can be measured as the relative tolerance (or buffer zone, Samia et al. 2017) that is the distance the animal can bear after recognizing the

approaching object (i.e. AD – FID, Fig. 3):

$$\text{Habituation by \%: } (1 - (AD_u - FID_u)/(AD_r - FID_r)) * 100$$

When this applies to the Eurasian red squirrels, habituation to human is 1.46 times more important compared to the reduction of vigilance (Table 3). Increased boldness to the predator, on the other hand, should result half from the decreased vigilance and half from habitation (probably via dogs, see above discussion). Unexpectedly, the relative tolerance to the control (novel object) was decreased in urban habitat (Table 3), which is probably due to neophobia in urban individuals (see above discussion). These calculations are based on the simple Euclidian distance, but in reality, for example, the same 5 m distance (assuming the relative tolerance) would be different when the object is 10 m or 30 m far from the animals: actual tolerance should be higher when animals can bear 5 m when the initial distance was 10 m. We could take such relative distance into account by log-transforming the data: that is, a higher weight when the approaching object is closer. The results changed slightly but more or less similar (Table 3: human habituation was two times more important and predator habituation was 1.3 times more important compared to reduced vigilance). Appropriate calculations should be based on animal cognitive systems and further analytical development is certainly required. Nevertheless, this method has a great potential to quantitatively assess the relative roles of decreased

vigilance and habituation on animal boldness. The simple measurement using the same unit (i.e. distance) also allows a meta-analysis for a wide range of urban animals.

The present approach also contributes to the management of urban animals. It is often perceived that increased boldness in urban animals can increase the vulnerability to novel predators, such as domestic cats, and novel threats, such as vehicles (Geffroy et al. 2015). This should be true when the increased boldness is largely due to decreased vigilance (Scenario I in Fig. 1). However, when the boldness reflects risk assessment (Scenario II in Fig. 1), urban animals can probably avoid such negative effects. Measuring boldness only with FID to human approach, the traditional method, cannot distinguish these cases and may result in inappropriate management actions. In spite of wide applicability and long history of flight distances, very few studies have employed both FID and AD toward multiple approaching objects. Our framework is promising to better understand adaptation to urban environments, as well as managements of urban wildlife.

Legends of figures and tables

Figure1

Predicted scenarios (I, II, III) of increased boldness due to the decreased vigilance and habituation to a human in urban animals. The x-axis represents categories of potential threats; risk levels of each threat are predicted as Human < Novel object < Predator. Y-axis represents alert distance (AD) and flight initiation distance (FID) towards approaching three types of potential threats. The circle and triangle symbols illustrate the degree of flight distance of individuals in non-urbanized areas and urban areas respectively. The scenario I) indicates decreased vigilance occurs without habituation to human due to the low predation risk. AD and FID would be decreased in urban areas, while FID towards each threat would not differ. Scenario II) indicates individuals habituate to human but vigilance is not decreased. In this case, the AD would not differ between urban and non-urbanized areas, and FID towards non-human threats would not also differ between two areas. On the other hand, FID towards human would shorter in urban than non-urbanized areas. Scenario III) indicates decreased vigilance habituation to a human occur simultaneously throughout the urbanization. In this case, AD and FID would be decreased in urban areas, and variation among FID towards each threat would be significant in urban areas.

Figure 2

The result of alert distance (AD) and flight initiation distance (FID) of urban and rural red squirrels towards each object. Means and standard deviations are shown. Sample size of AD of urban squirrels is 46 (human = 17, fox = 18, novel object = 11) and rural squirrels is 35 (human = 12, fox = 14, novel object = 9). The sample size of FID of urban squirrels is 78 (human = 24, fox = 29, novel object = 25) and rural squirrels is 59 (human = 25, fox = 19, novel object = 15).

Figure 3

Illustration of the concept of differentiating vigilance and habituation to human using AD and FID between urban and rural habitats. The relative tolerance can be calculated as $(AD - FID)$. Using this concept, the percentage of decreasing vigilance can be calculated as $(1 - AD_u/AD_r) * 100$. On the other hand, the percentage of habituation should be calculated as $(1 - (AD_u - FID_u)/(AD_r - FID_r)) * 100$.

Table 1. The result of ANOVA of alert distance (AD) and flight initiation distance (FID), comparing between urban and rural squirrels towards each object (human, fox, and novel object).

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

Table 2

The result of Turkey's multiple comparisons of flight initiation distance (FID) towards three objects between urban and rural habitats.

	Mean difference	95% Confidence interval		adjusted p value
		Lower bound	Upper bound	
Rural:Fox – Rural:Novel	3.014	-3.775	9.803	0.793
Rural:Human – Rural:Novel	0.504	-5.915	6.923	0.999
Rural:Human – Rural:Fox	-2.51	-8.492	3.472	0.83
Urban:Fox – Urban:Novle	-1.642	-7.001	3.722	0.949
Urban:Human – Urban:Novel	-8.25	-13.867	-2.633	< 0.001
Urban:Human – Urban:Fox	-6.607	-12.031	-1.183	<0.01
Urban:Human – Rural:Human	-14.001	-19.623	-8.389	< 0.001
Urba:Fox – Rural:Fox	-9.909	-15.71	-4.107	< 0.001
Urban:Novel – Rural:Novel	-5.252	-11.671	1.167	0.176

Fig1.

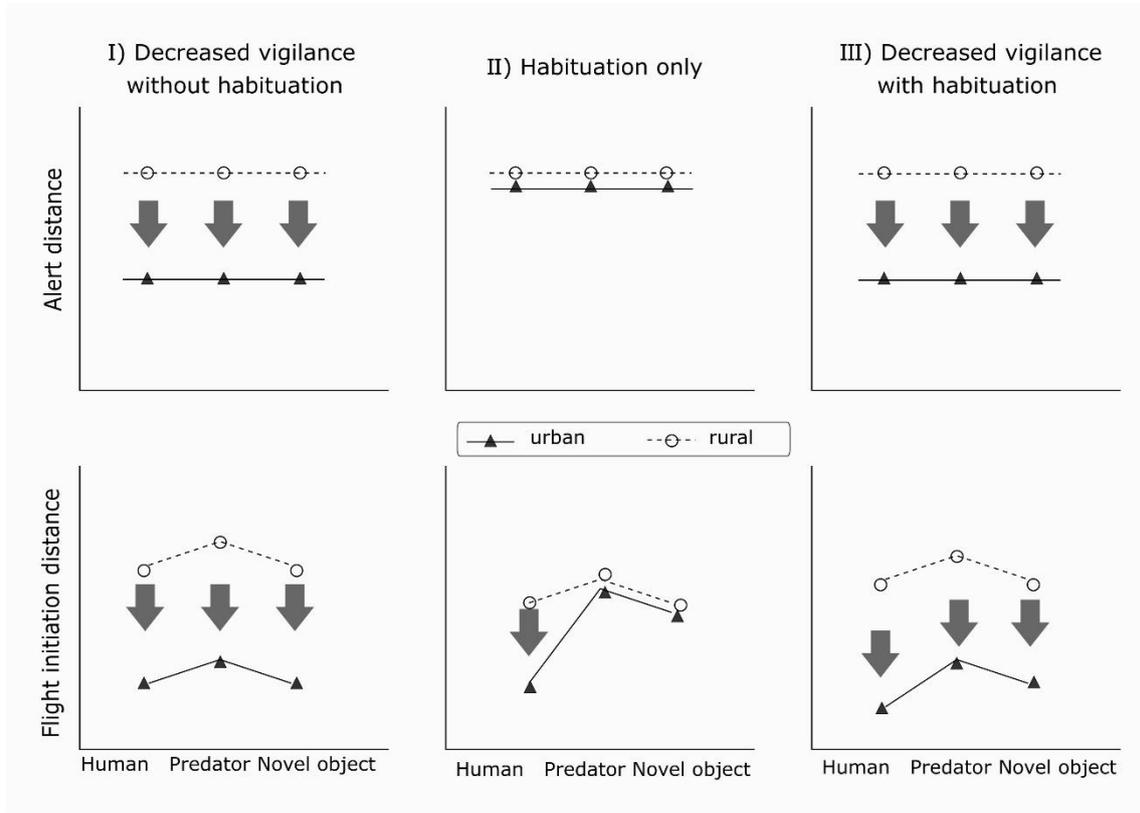


Fig. 2

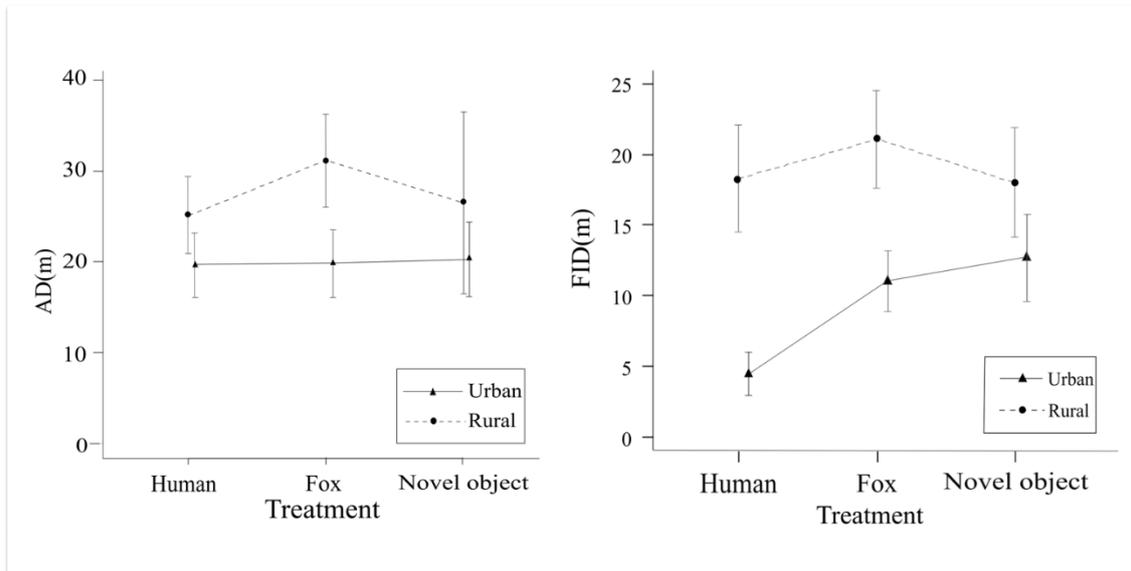
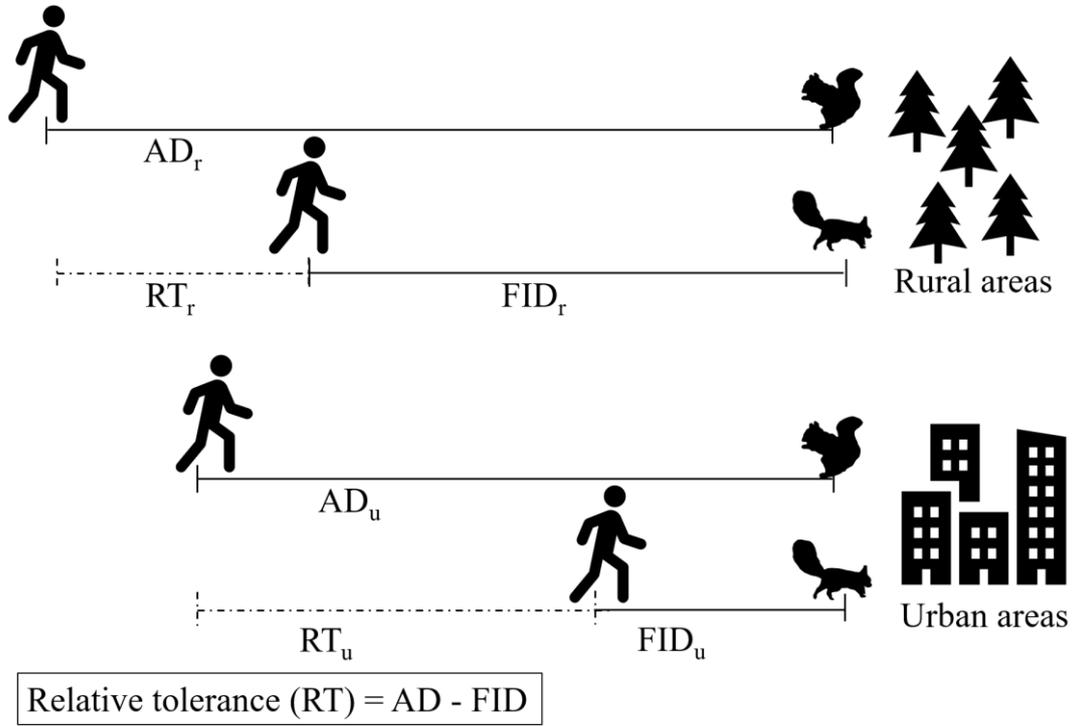


Fig. 3



CHAPTER 5

General Discussion



This thesis aimed to explore the behavioral adjustment of animals towards urbanization using Eurasian red squirrels by looking at several behavioral aspects. First, urbanization reduced the flight initiation distance (FID) and its seasonal variation of Eurasian red squirrels (Chapter 2). The result suggests that low predation risk and continuous human activity such as year-round supplemental feeding influence anti-predator behavior. Next, I compared various behaviors (e.g. behavioral indicator of boldness, activity, aggressiveness, exploration) between urban and rural habitats (Chapter 3). While most behaviors were not significantly different, human-related behaviors (i.e., VED and number of mobbing calls towards human) were clearly modified in urban squirrels. Finally, the last study showed that urban squirrels succeeded in modulating flight distances in response to different threatening conditions: especially, FID towards human was two times shorter than FIDs towards other threats (Chapter 4). Taken together, the present thesis revealed that behavioral changes in urban squirrels are only related to human responses, while they maintain basic behavioral characteristics or personality. Since most previous studies have examined behavioral shifts by human-related responses, such as FID, reported general trends (e.g. bolder, more aggressive in urban animals) may merely represent specific behaviors toward humans, not true trends. This thesis highlighted the importance of multiple behavioral assessments including the responses

towards non-human objects in order to understand the animal's adaptation to the urbanization. In this section, I discuss about the adjustment of animals to urbanization and its implication for conservation/management in the city.

Importance of evaluating multiple behaviors in non-avian species

In the last decade a growing number of literatures have been documented the behavioral shifts in a range of species (but highly biased to avian species), which provided us general predictions on how animals behaviorally adjust to the urban environments: e.g. becoming bolder, more explorative, and aggressive. Nonetheless, results of this thesis using Eurasian red squirrels which is a small mammal detected only shifting in human-related behaviors, while more than ten behaviors were not significantly different. However, some of my results are supported by current literatures using mammals: for example, Merriam's kangaroo rat *Dipodomys merriami* represented the lack of urban-rural differences in boldness and aggressiveness (Hurtado et al. 2017). In addition, Lyons et al. (2017) reported decreased activeness, exploration, and stress response in urban Eastern chipmunk *Tamias striatus* compared to conspecifics in non-urbanized habitats, which were opposite patterns of general predictions. These findings indicate that behavioral responses towards urbanization varies depending on species and taxa. In the case of red

squirrels, human presence probably has an outstanding effect compared to other species and other environmental factors because squirrels often have affinity relationships with human (Thoringtone et al. 2013). On the other hand, avian species might be influenced more by non-human environmental factors such as resources, artificial noise, and light.

Why do squirrels survive in urban areas?

Historically, arboreal squirrels have been thought to be sensitive to anthropogenic environmental disturbances such as forest fragmentations as well as urban developments because they are forest-dependent species (Verboom & Van Apeldoorn, 1990; Delin et al. 1999; Koprowski 2005). However, currently some Sciuridae such as Eurasian red squirrels have been colonizing urban areas across the northern Eurasian continent (Fey et al. 2015; Uchida et al. 2015; Jokimäki et al. 2017; Hämäläinen et al. 2018). One of the differences between fragmented forests and urban areas should be food abundance. Jokimäki et al. (2017) suggested that supplemental feeding may be one of strongest factors attracting squirrels to urbanized area in Finland. Squirrels are popular among people in many countries: squirrels can be good targets for environmental education and recreation (Thorington et al. 2013) and people often provide food. Low predation risk is

another potential difference between urban area and disturbed forests. Large carnivores and raptors, the main predators of squirrels, are rarely observed in urbanized areas, whereas they often survive even in fragmented forests (e.g., Uchida, personal observation). Although there are novel threats (e.g. cars and domestic cats) in urban areas, my result showed that urban squirrels succeeded in reacting different risk conditions.

In the case of Tokachi region, landscape structure may also play a key function for squirrels to invade into urban areas. There are plenty of isolated forests either in rural and urban areas, which could be used as temporal habitats and stepping-stone during dispersal stages. Especially, wind break forests that go through from rural to urbanized areas are likely to be effective corridors for squirrels.

Squirrel's colonization to and survival in urban areas may be also attributed to their intrinsic characteristics. Behavioral plasticity would play an important role in dealing with urbanization (Sol et al. 2013) and it is shown that the increased boldness of fox squirrels in urban areas is due to learning, a specific type of behavioral plasticity, rather than genetic adaptation (McCleery 2009). Some of my results also indicate high behavioral plasticity against different situations, such as changing behavioral tendency

across time in MIS test (Chapter 3) and FID variations towards different threat conditions (Chapter 4).

The potential of long-distance dispersal should be also considered. There are some evidences that squirrels can disperse more than ten kilometers during the natal dispersal stage (Wauter et al. 2010; Hämäläinen et al. 2018). Fey et al. (2015) also reported that juvenile squirrels frequently crossed either big or small roads, although adults rarely crossed such grey matrices. In my field site, my colleagues also revealed that the red squirrels in two areas (urban and rural) are genetically similar, and thus indicates active gene flow among various habitats (Watanabe, Uchida et al. unpublished data). This is probably one of the reasons why most behavioral traits were not diverged (Chapter 3).

Taken together, squirrels may perform even better in urbanized habitats, as suggested in some other animals (e.g. Rebolo-Ifran et al. 2015; Lyons et al. 2017). In fact, I found no significant difference in fecal cortisol levels, an indicator of stress responses, between urban and rural squirrels, and found that the body weight of urban squirrels was rather little heavier (Shimamoto, Uchida et al. submitted). Although it is not easy to conclude that squirrels are well adjusting to the urban environment, I believe that red squirrels are relatively robust to the urbanization.

Implications to wildlife management

The most relevant achievement of the thesis to conservation or management is probably the framework for differentiating the effects of reduced vigilance and habituation to humans on increased boldness (Chapter 4). There are many situations where managers wish to promote animal habituation for minimizing their stresses induced by humans (Lowry et al. 2013). Habituation, however, has some risks. For instance, it is pointed out that habituation to human increases vulnerability towards predation because this can be transferred to other potential predators (Geffroy et al. 2015; Blumstein 2016). This idea was partly supported by the study of fox squirrels *Sciurus niger* that the population in urbanized area were bolder to both human and predator cues compared to nonurbanized areas (McCleery 2009), although such empirical study is still lacking. If this happens, human presence is likely to change prey-predator interactions, ultimately resulting in deleterious impacts on local ecosystems. The novel framework proposed in this thesis can evaluate if such transfer of habituation occurs. Many previous studies have assessed the change in boldness with human-induced responses, which can mislead the context-dependent behavioral flexibility of urban animals important for survival.

Some other findings also provide important insights into mitigating human-wildlife conflict in urbanized areas. Remarkable reduction of fearfulness towards human in urban individuals would be one of the key factors for traffic accidents (i.e. road kill) and aggressions to human (Fuentes & Gamerl 2005; Warne et al. 2010). My results showed that urban animals can flexibly change behaviors, which in turn suggests that it is possible to change animal behaviors to avoid human as threatening objects. Levey et al. (2009) showed that Northern Mockingbirds *Mimus polyglottos* live in human residential areas rapidly identified the potentially harmful humans who exposed threatening behaviors and increased responsiveness towards them. In short, we might be able to control animal behaviors by differently interacting with them. Understanding the processes of animal habituation and sensitization to human could significantly contribute to the mitigation of human-wildlife interactions. There is a great potential to collaborate with researchers in animal cognition and psychology to deepen our understanding of how animals respond to human for future works.

Finally, I would like to point out that many findings in this thesis can be also applied to non-urbanized areas as long as human utilization is intense. Growing probability of human-wildlife interaction is predicted to be inevitable because outdoor activity and ecotourism is becoming more and more popular (Balmford et al. 2015). For instance, over

eight billion people visit natural terrestrial areas per year (Balmford et al. 2015). If animals in such areas habituate to humans but cannot well recognize different predators, they will strongly suffer from predation because natural predators are still exist unlike urban areas. Therefore, understanding of animal reactions towards human in urbanized areas will be also useful for management and conservation under non-urbanized ecosystems.

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