Anti-predator strategy of frogs against snakes:

adaptive decision making for alternative use of fleeing and immobility

NOZOMI NISHIUMI

2015

CONTENTS

ABSTRACT

Introduction

In anti-predator mechanisms, when the prey is located within the mutual perceptual field of prey and predator, prey responds by reducing the probability of successful predation. In this situation, prey animals often engage in secondary defense phase, which requires appropriate decision making of using anti-predator tactics. Most of cryptic prey animals are considered to use two types of anti-predator behavior as secondary defense; one is immobility, which is a motionless state of prey to enhance crypsis against visually hunting predators, and another is fleeing to increase distance from predator. In the present study, I focused on immobility and fleeing and conducted field and experimental studies to examine decision making for the use of these tactics in frogs, which are known to use immobility and fleeing against snakes to avoid predation. I considered two situations according to the status of predatory sequence: when snakes have not detected prey (Chapter 1) and when snakes have detected prey (Chapters 2 and 3). In both situations, I first experimentally examined how frogs switch these tactics. Then, I examined how the switching affects survival of the frogs. Finally, based on the results of these experiments, I propose several factors that should be included in the future theoretical models of optimal anti-predator strategy.

Methods

The subjects are a ranid frog, *Pelophylax nigromaculatus*, and a colubrid snake, *Elaphe quadrivirgata*, both of which were collected from paddy field in Kyoto

Prefecture, Japan. In Chapter 1, first I examined behavior of a frog that was introduced in an arena with a snake that has not detected the frog. I observed decision of the frog about choosing immobility or fleeing against the snake, focusing on distance between them. Then, I examined effectiveness of its decision. I observed predatory response of a snake against a frog in immobile or moving state at two different distances between them. In Chapter 2, I manipulated movement of a frog to induce a snake to detect the frog, and I examined behavior of the frog against the snake that has detected the frog. Then, I examined effectiveness of its decision by observing predatory response of a snake to immobile and moving frogs. In Chapter 3, I first examined duration of predatory event between frogs and snakes in nature. Then, I experimentally examined factors that affect successful escape of frogs in this duration, especially focusing on strike behavior of snakes in close quarters.

Results

In Chapter 1, frogs initially exhibited immobility, when snakes were moving at a long distance, and then switched from immobility to fleeing at a shorter distance even when snakes had not detected them. On the other hand, snakes at long distance detected only fleeing frogs, whereas snakes at short distance detected both immobile and fleeing frogs. In Chapter 2, frogs exhibited immobility against a snake that has detected the frog. To the immobile frog, snakes approached more slowly than to the moving frog. In the situation of a single frog, snakes eventually reached the frog and struck it regardless of its behavioral state. However, in the situation of two frogs, the immobile frog survived because snakes are distracted to a moving frog. In Chapter 3, it was confirmed that the

predation events between frogs and snakes are settled within a few seconds because there are refuges for frogs around the predation event. In the experiment, video analysis revealed that snakes are not able to change the trajectory of strike after initiating it and are not able to move in a split second after the strike behavior.

Discussion

Chapter 1 showed that the ability of snakes to detect motionless frogs depends on the distance, suggesting that the distance-dependent switching can be considered an adaptive strategy of the frog. However, a previous model predicts that cryptic prey should flee immediately on seeing a predator or not flee until being detected by the predator. To explain this discordance, I propose two new factors that affect the decision of switching from immobility to fleeing. In Chapter 2, it was demonstrated that even if immobility may lose its cryptic advantage when predator has detected the prey, immobility has another function of increasing latency of predator to attack. The increased latency heightens the probability of emergence of other prey within a perceptual area of predator, and then, immobile prey would be able to survive by distracting the predator to the new prey. In Chapter 3, it was suggested that snakes are not able to move immediately after frogs evaded their strike movements. In addition, frogs are able to evade the strike of snakes at least with approximately 10 cm separation. Thus, keeping immobile to induce strike of snake would result in successful escape.

Conclusion

In theoretical biology, it has been assumed that the optimal timing of switching from immobility to fleeing should be triggered by the detection of predator. However, in the present study, it was demonstrated that timing of switching from immobility to fleeing does not necessarily depend on the detection of predator. Although the present study was conducted in a simplified environmental condition, and examinations in more natural setting would be required, I anticipate that incorporating findings in the present study will contribute to better understanding of the anti-predator strategy of animals in the real world.

GENERAL INTRODUCTION

Predation avoidance is an essential process for prey to survive, and thus, prey has evolved various traits to overcome predator (Dawkins and Krebs 1979). In anti-predator mechanisms, prey responds by reducing the probability of successful predation when the prey is located by predator within the mutual perceptual field (Brodie et al. 1991). In this situation, prey animals often engage in a secondary defense phase, which requires appropriate decision making of using anti-predator tactics (Edmunds 1974). The study of decision making of using anti-predator tactics in secondary defense is essential for understanding strategic components of secondary defense, and thus, the study has been developed in both theoretical and empirical research fields (Ydenberg and Dill 1986; Ducey and Brodie 1983).

In escape theories, the prey may not always perform anti-predator behavior immediately on seeing the predator, even if the probability of escaping from the predator is reduced by this delay (Ydenberg and Dill 1986). The mechanism underlying the delay is that the prey must often face trade off between the risk of being eaten and other potential benefits, such as food acquisition (Ydenberg and Dill 1986). The prey initiates anti-predator responses when the predator comes close to a distance where the cost of being eaten exceeds other potential benefits. Following the economic escape model by Ydenberg and Dill (1986), optimal escape models have been developed, which predict escape decision based on economic considerations about effects of predation risk, current fitness, and cost of escaping (Cooper and Frederick 2007). In both economic and

optimal escape models, fleeing is considered as a representative anti-predator behavior (Ydenberg and Dill 1986; Cooper and Frederick 2007). However, fleeing is not necessarily a single option of anti-predator behavior against approaching predator in some prey animals that have a variety of anti-predator behaviors (Burger 1974; Caro 2005; Wasson and Lyon 2005; Ford and Reeves 2008; Gerald 2008; Toledo et al. 2011). Thus, when these prey animals engage in anti-predator tactics, they must choose the most appropriate response among multiple anti-predator behaviors to maximize survival.

Broom and Ruxton (2005) introduced an optimal switching model between secondary defense tactics. They assumed cryptic prey that has two types of anti-predator behavior; one is immobility, which is a motionless state of prey to enhance crypsis against visually hunting predators (Edmunds 1974; Endler 1991; Toledo et al. 2011), and the other is fleeing to increase distance from predator (Endler 1991). It is assumed that if prey has a sufficient head start of fleeing, prey will be able to avoid predation, either by outrunning the predator or by safely reaching a refuge (Broom and Ruxton 2005). However, fleeing from the predator will in most cases alert it to the presence of the prey individual. The predator may respond to this detection with attack, which may be successful. Thus, there may be a countervailing pressure for the prey to use immobility. This behavior may allow the prey to survive because the predator may pass without recognizing the presence of the prey. However, immobility incidentally allows a predator to come closer, which reduces both the probability that the predator pass without recognizing the prey, and the probability of successful fleeing. Therefore, to survive, prey animals must achieve an appropriate balance between immobility and fleeing. Broom and Ruxton (2005) demonstrated that

the optimal strategy for cryptic prey is either fleeing immediately on seeing the predator or never initiating fleeing until when the predator has detected the prey. In addition to this prediction, they emphasize the importance of empirical studies that compare multiple tactics for clarifying optimal decision of tactic choice to understand the defensive strategy of prey (Broom and Ruxton 2005).

However, such empirical studies have not been carried out sufficiently. Predictions of escape theories have been confirmed and developed by experimental studies using real prey animals, mainly lizards and crickets (Cooper 1997; 2003; Cooper et al. 2003; Lagos et al. 2014). The repertories and effectiveness of anti-predator tactics against real predators have also been reported (Wasson and Lyon 2005; Ford and Reeves 2008; Miyatake et al. 2009; Toledo et al. 2011). However, in spite of the piles of studies reporting anti-predator tactics, studies that compare multiple tactics for clarifying optimal decision of tactic choice are scarcely explored (but see Ducey and Brodie 1983), especially in the view point for applying to the optimal tactic-choice model, such as the Broom and Ruxton model (2005).

The present study deals with decision making of tactic choice by comparing immobility and fleeing in frogs from empirical aspect. Frogs are known to be preyed upon by a variety of predators (Duellman and Trueb 1994). They are able to detect a predator by its movement and exhibit defensive behaviors, such as fleeing, immobility, puffing up their body, counterattack and secreting chemicals (Marchisin and Anderson 1978; Toledo et al. 2011). Among them, the most commonly observed defensive behavior is fleeing, followed by immobility (Toledo et al. 2011). Snakes are the most typical predators of frogs (Toledo et al.

2006). Especially active diurnal hunting snakes are known to detect prey mainly with visual cue (Mattison 1995). Such snakes may pass immobile frogs without recognizing them (Ducey and Brodie 1983). Therefore frogs that mainly use immobility and fleeing and active diurnal hunting snakes are suitable model animals for examining the switch of prey between immobility and fleeing during predator-prey encounter.

This thesis is divided into three chapters according to the phase of predatory sequence. Chapter 1 deals with optimal decision when predator has not detected prey and when predator has detected prey, and Chapter 2 and 3 deal with optimal decision when predator has detected prey. Chapter 2 focuses on a function of immobility that increases latency of predator to attack, and Chapter 3 focuses on optimal flight initiation timing for successful escape. In all chapters, I first experimentally examined how frogs switch these tactics. Then, I examined how the switching affects survival of the frogs. Finally, based on the results of these experiments, I propose several factors that should be taken into considerations for development of study of anti-predator strategy.

Chapter 1: Decision making before being detected by a predator snake

"Distance-dependent switching of frogs from immobility to fleeing"

1-1 Introduction

In the early stage of the predatory sequence, it is considered that many prey animals in secondary defense exhibit anti-predator behaviors that is effective against a predator that is searching for or approaching prey. A representative anti-predator behavior against a searching predator is immobility, which is a motionless state of prey to enhance crypsis against visually hunting predators (Edmunds 1974; Endler 1991; Toledo et al. 2011), and one against an approaching predator is fleeing (Endler 1991). When predator is searching for prey and has not detected it yet, it is assumed that if prey has a sufficient head start of fleeing, prey will be able to evade predation, either by outrunning the predator or by safely reaching a refuge (Broom and Ruxton 2005). However, fleeing from the predator will in most cases alert it to the presence of the prey individual. The predator may respond to this detection with attack, which may be successful. Thus, there may be a countervailing pressure for the prey to use immobility. This behavior may allow the prey to survive because the predator may pass by without recognizing the presence of the prey. Therefore, to survive, prey animals must achieve an appropriate balance between immobility and fleeing.

According to the Broom and Ruxton (2005) model, the optimal response of cryptic prey is either fleeing immediately on seeing the predator. In their model,

they assumed forestall fleeing and longer distance between prey and predator as factors that positively affect probability of successful escape, and immobile state and the longer distance as factors that positively affect probability of letting predator pass without detection. Thus, it was also predicted that initiating fleeing until when the predator has detected the prey is never optimal, because the prey thereby abandons the advantage of crypsis and initiates fleeing at a shorter distance than that of the immediate fleeing.

In Chapter 1, I first examined whether frogs switch these tactics optimally, as predicted by the theory of Broom and Ruxton (2005). Then, I experimentally examined how the switching affects survival of the frogs. Finally, based on the results of these experiments, I propose several important factors that should be included in the future theoretical models of optimal anti-predator strategy.

1-2 General Methods

Study organisms

The subjects were a ranid frog, *Pelophylax nigromaculatus*, and a colubrid snake, *Elaphe quadrivirgata*. *Pelophylax nigromaculatus* is a pond frog densely distributed over a large part of East Asia, including Japan, Korea, China, and the Amur Basin of Russia (Maeda and Matsui 1999; Shinohara 2007; Zhang et al. 2008). *Elaphe quadrivirgata* is widely distributed in Japan and is a dietary generalist, mainly feeding on frogs including *P. nigromaculatus* (Mori and

Moriguchi 1988; Kadowaki 1996; Goris and Maeda 2004). Because *E. quadrivirgata* is diurnal, it is presumed that the snake detects prey mainly by visual cues (Ota 1986).

A total of 124 frogs were used for the experiments. All frogs were collected from Kyoto Prefecture, Japan. They were housed individually in clear plastic terraria (130 × 210 × 160 mm). The floor of each terrarium was slightly inclined, and the terraria contained water that covered half of the floor. The terraria were kept under the natural ambient photoperiod and at air temperature during May-September in Kyoto. During October, the terraria were kept in a laboratory where air temperature was maintained between 25°C and 30°C. Illumination was provided by sunlight. All frogs were used for experiments within two weeks after they were captured. Twenty-three snakes were used for the experiment (19 and 4 snakes from Kyoto and Tokushima Prefectures, respectively). They were collected from areas sympatric with *P. nigromaculatus*. Snakes were housed individually in clear plastic terraria (405 × 265 × 200 mm) containing glass vessels with water, paper floor and a few pieces of broken plant pots as shelter. The terraria were kept in a laboratory where air temperature was maintained between 25°C and 30°C. Illumination was provided by sunlight. All snakes were fed two or three frogs per week. After the experiment, all frogs were eventually fed to these snakes, except for 15 frogs that were eaten during the experiment, and snakes were basically released at the site of capture.

Experimental apparatus

The test arena, measuring 1175 × 452 × 425 mm, was made of clear glass

panels, and set on center place of an experimental room, measuring 4 × 2 × 2 m. The arena was divided into two compartments (indoor and outdoor spaces) by a white plastic board (Fig. 1). One edge of this board was attached to a wall of the arena via hinges so that the board could be lifted up by being pulled up with a string. All trials were filmed with a video camera (Canon IVIS HV30) by means of a mirror.

1-3 Experiment 1: examination of the effects of snake movements and distance between frogs and snakes

In this experiment, I examined factors that affect the occurrence of immobility and fleeing in encounters between predator and prey. Experiment 1 comprised of two parts: experiments 1A and 1B. Experiment 1A was conducted to examine the effects of snake movement on the behavior of frogs. Experiment 1B was conducted to examine the effects of distance between a frog and a snake on the behavior of the frog.

1-3-1 Methods

In Experiment 1A, the outdoor space of an arena was divided into two compartments: prey and predator compartments, by a clear plastic partition that contained many small holes (Fig. 1). Experiment 1A consisted of three sessions:

first I observed the behavior of a frog without a snake (Control session-1; CS1), then I observed it with the presence of a snake (Experimental session; ES), and finally I conducted a control session again (Control session-2; CS2). The same frog was used repeatedly throughout CS1, ES and CS2. The duration of each session was 1 hour, and the interval between two successive sessions was 1 day. In ES, I introduced a snake and a frog into the indoor space and the prey compartment of the outdoor space, respectively. Ten minutes after introducing the frog, I lifted the door and recorded the behavior of the snake and frog with the aid of a video camera for 1 hour. The distance between a frog and a snake at the start of ES was at least 400 mm. During this session, both frog and snake were allowed to utilize visual and chemical cues through the partition board. In CS1 and CS2, I introduced only a frog into the prey compartment of the outdoor space and recorded its behavior in the same way as in ES. I conducted 18 trials, and each trial contains these three types of session. Eighteen frogs and two snakes were used in the experiment. Mean body mass of the frogs was 5.7 g (range 2.0-19.2 g). Body mass of the snakes was 361 g and 478 g, and their snout-vent length was 1059 mm and 1230 mm.

I used C-trax software for analyzing the speed of the locomotive movements of frogs in the video data. C-trax is an open-source program for estimating the positions and orientations of many individual walking flies over long periods of time. It was released by the California Institute of Technology and is designed to allow high-throughput, quantitative analysis of behavior in freely moving flies (http://ctrax.sourceforge.net/). Because the minimum speed of locomotive movements that I was able to recognize was 40 mm/s, I discarded movements whose speed was less than 40 mm/s as noise, and then I counted the number of

the frog movements. I divided the ES into two periods: when the snake remained motionless (ES-ml) and when it was moving (ES-mv), and then I analyzed the data using the multiple comparison procedure following Friedman test (Siegel and Castellan 1988) applying treatment (CS-1, CS-2, ES-ml and ES-mv) as independent variable and the rate of frog movements as dependent variable.

In Experiment 1B, I introduced a frog and a snake into the indoor space and the outdoor space, respectively, separated from each other by at least 800 mm. The arena did not have a partition, so that the snake was allowed to approach the frog. Ten minutes after introducing them, I lifted the door and recorded their behavior with the aid of a video camera for 1 hour. I recorded the timing of the flight initiation of frogs in relation to behavior of snakes and distance between a frog and a snake. I defined the following three responses of *E. quadrivirgata* to frogs. Phase 1: Orienting – a sudden displacement of the head, head and neck, or the anterior part of the body in the direction of the prey. The position of the whole body does not change. Phase 2: Straight approaching – slow or rapid locomotion straight toward the prey. Phase 3: Striking – opening the jaws and projecting the head, head and neck, or the anterior part of the body rapidly toward the prey. I determined whether snakes detected frogs based on behavior of the snakes. However, it is difficult to judge the occurrence of detection based on the orienting behavior because snakes orient not only to frogs but also to many other kinds of objects. Thus, I considered orienting as only an indicator that snakes detected some object(s), and I used straight approaching and striking as an indicator that snakes detected the frogs. Fifty frogs and 13 snakes were used. Mean body mass of the frogs was 4.8 g (range 0.7-16.6 g). Mean body mass and snout-vent length of the snakes were 243 g (range 25-500 g)

and 923 mm (range 427-1270 mm), respectively. No frogs were used more than once.

1-3-2 Results

In the ES of Experiment 1A, the snake initially remained motionless. Once the snake started moving, it kept moving almost continuously until the end of the session. Mean ± SD of duration of ES-ml and ES-mv was 18 ± 14 min and 43 ± 14 min, respectively (n = 18). During the ES, the mean number of movements of the frogs was 49 (range: 0-421 g), and most of the movements were observed while the snake remained motionless (mean = 48). The treatments significantly affected the rate of frog movements (Friedman test: $\chi^2 = 21.3$, df = 3, $P < 0.001$). There were no significant differences in the rate of frog movements (the number of movements / hour) among CS-1, CS-2, and ES-ml (multiple comparison: each $|R_u-R_v| < 20.43$, each $P > 0.05$; Fig. 2). The lack of a significant difference between CS-1 and CS-2 indicated the absence of acclimation effects. The rate of frog movements when the snake was moving (ES-mv) was significantly lower than that during CS-1, CS-2 and ES-ml (multiple comparison: each $|R_u-R_v| \geq 20.43$, each $P < 0.05$; Fig. 2).

In Experiment 1B, after the snake started moving, it did not approach the frog directly, but rather crawled around the arena without orienting to the frog. Consequently, the snake shortened the distance between the frog and itself, and in all cases the frogs fled before the snake reached them, and the snake showed neither orienting, straight approaching nor striking before the flight initiation of

the frogs. Mean ± SD of the distance between the snake and the frog when the latter exhibited the first fleeing was 80 ± 67mm (n = 50; Fig. 3).

1-4 Experiment 2: examination of the effectiveness of immobility in relation to distance

In Experiment 1, frogs switched from immobility to fleeing according to their distance from snakes. This suggests the increase of predation risk with decreasing distance to a predator. I assumed that the probability of detection of motionless frogs by snakes might increase as the distance between them becomes short. To test this assumption, I examined the effectiveness of immobility against snakes at two different distances.

1-4-1 Methods

I examined the responses of snakes against a motionless and a moving frog at two different distances: short distance (0-100 mm) and long distance (400-800 mm). The responses of snakes were defined as Experiment 1B.

I introduced a frog and a snake into the indoor space and the outdoor space, respectively, separated from each other by at least 800 mm. The arena did not have a partition, so that the snake was allowed to approach the frog. Ten minutes after introducing them, I lifted the door and recorded their behavior with

the aid of a video camera for 1 hour. After the snake started moving, the snake usually explored the arena, shortening its distance from the frog. In the test of snakes against a motionless frog, I observed the snake's behavior toward a motionless frog when the snake was crawling within the range of 400-800 mm (session of motionless frogs at long distance; session-ml-L) and within the range of 0-100 mm (session of motionless frogs at short distance; session-ml-S). In the test of snakes against a moving frog, I observed the snake's behavior toward a moving frog when the snake was crawling within the range of 400-800 mm (session of moving frogs at long distance; session-mv-L) and within the range of 0-100 mm (session of moving frogs at short distance; session-mv-S). I terminated the sessions when the snake moved out of the distance range without showing straight approaching or when the snake struck the frog.

According to the results of Experiment 1B, within the long distance range, frogs usually exhibited immobility and did not voluntarily move, and within the short distance range, frogs usually exhibited fleeing as a response to an approaching snake. Thus, I used frogs without manipulation in session-ml-L and session-mv-S. However, I needed to manipulate frogs to initiate moving in session-mv-L, and to inhibit moving in session-ml-S. To induce the frogs to move within the long distance range, a string was tied around the bellies of the frogs. By pulling the string from outside of the arena, I induced the frogs to perform locomotive movement similar to voluntary jumping. After the snake reached the long distance range, I pulled the string basically once every five seconds until the snake struck the frog. On the other hand, to prevent frogs from fleeing within the short distance range, I lowered their body temperature because frogs are not able to move at low body temperature. I soaked the frogs in ice water for 15 min

and then put them on an ice pack (100 mm × 100 mm × 10 mm) on the floor of the arena and kept them there during the session.

In session-ml-L, 20 snakes and 20 frogs were used. Mean body mass of these frogs was 5.0 g (range 4.3-12.7 g). Mean body mass and snout-vent length of these snakes were 219 g (range 25-500 g) and 915 mm (range 427-1270 mm), respectively. In session-ml-S, nine snakes and nine frogs were used. Mean body mass of these frogs was 2.1 g (range 1.1-5.0 g). Mean body mass and snout-vent length of these snakes were 179 g (range 42-355 g) and 874 mm (range 530-1120 mm), respectively. In session-mv-L, seven snakes and seven frogs were used. Mean body mass of these frogs was 8.3 g (range 2.4-12.1 g). Mean body mass and snout-vent length of these snakes were 166 g (range 112-320 g) and 869 mm (range 720-1120 mm), respectively. In session-mv-S, 20 snakes and 20 frogs, which were the same individuals as those in session-ml-L, were used. No frog was used more than once, but seven snakes were used in both session-ml-L and session-mv-L, and in both session-mv-S and session-ml-S.

I analyzed the data of Experiment 2 using GLMM with binomial family, applying the occurrence of detection of the frog as dependent variable, the distance between the frog and the snake at the start of each session (long or short) as a fixed factor, and snake's ID as a random factor. The statistical package JMP (version, 8.0.2) was used for the GLMM analyses.

1-4-2 Results

In the test of snakes against a motionless frog, no snakes showed orienting, straight approaching or striking against the frog at the long distance. On the other hand, at the short distance all snakes approached the motionless frog slowly but not straightly while frequently flicking their tongues, and eventually contacted the frog with their snouts. Eight of the nine snakes then grasped the frog with their jaws (striking). The other snake did not exhibit striking and instead resumed crawling. The distance significantly affected the occurrence of predatory behavior of snakes (GLMM: Coefficient = 0.44, t = 12.21, $P < 0.0001$).

In the test of snakes against a moving frog, all snakes immediately exhibited orienting to the frog at the long distance, and then performed straight approaching and finally struck the frog (Table 1). On the other hand, against the moving frog at the short distance, although all snakes immediately exhibited orienting to it, only 70% of the snakes performed straight approaching and struck it. The remaining 30% of the snakes performed orienting but exhibited neither straight approaching nor striking, and then resumed crawling. The distance did not significantly affect the occurrence of predatory behavior of snakes (GLMM: Coefficient = - 0.15, t = -1.67, $P = 0.108$).

1-5 Discussion

The results of Experiment 1 suggest that frogs recognize the predation threat of a snake by its movement always before they are detected by the snake, which is the basic assumption of the model of Broom and Ruxton (2005). Nonetheless,

contrary to their predation, frogs switch from immobility to fleeing at an intermediate time between detecting a snake and being detected by it. The results of Experiment 2 demonstrated that immobility of the frog was effective for avoiding detection by snakes only at a long distance. When the snakes were positioned at a close distance, the frog was not able to avoid detection regardless of their anti-predator behaviors.

In the model of Broom and Ruxton (2005), it was demonstrated that the optimal strategy for prey is either fleeing immediately on seeing the predator or never initiating fleeing until when the predator detects the prey. The former strategy has the advantage that the prey can initiate fleeing at the maximum distance, and the timing of the initiation of fleeing is before the prey is detected, when the predator may not be able to respond to it immediately. On the other hand, the latter strategy has an advantage that the predator may pass without detecting the prey. In addition, Broom and Ruxton (2005) demonstrated that it is never optimal for prey to use immobility first, and then initiate fleeing after waiting for the predator to reach at a certain distance but before being detected. In this “inappropriate” strategy, the prey abandons the advantage of crypsis and initiates fleeing at a shorter distance than that of immediate fleeing. However, in contradiction to this model, the frogs in Experiment 1B responded with the “inappropriate” strategy: they remained motionless when they first noticed the moving snake, and then they initiated fleeing at a certain distance before the snake obviously detected them. There are at least two possible explanations for this unexpected result.

First, when frogs initiated fleeing, although snakes had not detected frogs,

the future detection may have been no longer avoidable if the frogs remained motionless. The model of Broom & Ruxton (2005) is based on the assumption that there is still possibility that the predator will pass by the prey without detecting it if the predator has not detected it. However, if the predator engages in a searching mode that enables it to eventually detect the prey within a certain range, the circumstance of unavoidable future detection would occur. Immobility is a cryptic tactic that is effective against predators using a visual sense, and it may not work to avoid detection by other senses, especially at a short distance. In the present experiments, snakes were able to detect immobile frogs within 100 mm distance even in the absence of the movement cue. It is well known that snakes have a keen chemical sense that relies on the vomeronasal organ (Jacobson's organ) and that they are able to detect prey with chemical cues alone (Halpern 1987; Wattiez et al. 1994). Thus, it is likely that *E. quadrivirgata* used some chemical cue(s) for detecting nearby frogs. Although chemical cues are not effective to locate the exact position of remote frogs, intensive local search using chemical cues would enable snakes to eventually detect the prey in the vicinity. Therefore, even when snakes have not detected frogs, the frogs should initiate fleeing at a distance that they are expected to be detected sooner or later. The occurrence of the intensive searching mode at short distance, which leads to definite detection of prey, may be one of possible explanations of the discordance between my results and the prediction of Broom and Ruxton (2005).

Second, the relationship between the distance and the probability of successful escape by fleeing may not be simple. The theory of Broom and Ruxton (2005) is based on a presumption that the probability of successful escape by fleeing decreases as the distance between the prey and the predator

decreases because they simply considered that the function of fleeing is to increase the distance between prey and predator. However, contrary to this presumption, close distance may add other functions on fleeing. For example, when prey initiates fleeing at a shorter distance, the angle between the longitudinal axis of the head of the predator and the line from the predator to the prey changes rapidly. Consequently, the predator may not be able to keep tracking of the moving prey (Howland 1974). Moreover, the predator may not be able to immediately recognize the moving object at close proximately as prey or the predator may be frightened by the sudden movement of the prey (Gamberale-Stille et al. 2009). Indeed, 30% of the frogs in Experiment 2 that initiated fleeing at the short distance did not induce immediate attack of the snakes. Therefore, initiating fleeing at close distance may provide an additional defensive function, resulting in the lower predation risk than fleeing at intermediate distance. This may be the reason why the frogs did not flee immediately when they recognized the snakes, but subsequently initiated fleeing before being detected.

In summary, I propose two new viewpoints for understanding interplay between predator and prey. First, at a short distance, some predators switch to intensive searching mode with the aid of additional sensory cues, which leads them to eventually detect the prey. Against such predators, adaptive response of the prey in a short distance is to start fleeing even before the predator detects it. This presumption has not been considered in the model by Broom and Ruxton (2005), in which prey still has chance to induce the predator pass without detection while predator has not detected it. Second, close distance can create an additional defensive function of fleeing. I call this effect “close-quarters effect”.

Close-quarters effect would be apply to not only frog-prey and snake-predator system but also many other animals. For example, as a butterfly opens its wings with eyespots against predator in order to flee, the deimatic impact would be enhanced at short distance (Gamberale-Stille et al. 2009; Vallin et al. 2005). In studies of anti-predator strategy, the probability of escaping predation by fleeing has been simply assumed as a monotonically increasing function of distance between a prey and its predator. However, it is highly likely that another distance-dependent effect, such as close-quarters effect, would partly change the shape of the function from monotonically increasing to convex upward, resulting in higher escape probability of fleeing in proximate distance.

Chapter 2: Decision making after being detected by a predator snake, part 1

"Immobile tactic of frogs for increasing latency of predator to attack"

2-1 Introduction

In Chapter 1 I dealt with a situation that prey has not been detected by predator. In this chapter I deal with a situation that prey has been detected by predator. In this situation, according to the currently accepted function of immobility, that is enhancing crypsis, prey should select fleeing rather than immobility because the cryptic function of immobility is no longer effective under being detected by predator (Endler 1991; Broom and Ruxton 2005; Ruxton et al. 2004). However, some prey animals often exhibit immobility instead of fleeing even when predator has detected them (Brodie et al. 1974). This implies that keeping immobility under being detected by predator has some kinds of advantage for predation avoidance. In this chapter, I focus on predators with two characteristics mentioned below and examine the possible advantages of immobility under being detected by predator.

First, some predators are known to stalk their prey with careful slow speed until they reach an optimal distance to initiate pursuing the prey (Van Valkenburgh 1985). If the prey initiates escape before they reach the optimal distance for pursuit, they would immediately initiate pursuing the prey. Thus, against such predators, escape behavior of prey would trigger the initiation of their pursuing; in other words, remaining immobile has an effect to increase latency of predators to initiate pursuing.

Second, some predators are more strongly attracted to moving prey as a

target than immobilized prey (Jackson and Pollard 1996; T. Miyatake et al. 2009; Chapter 1). Against such predators, remaining immobile has effect to reduce their predatory interest (Miyatake et al. 2004).

I assumed a situation where multiple prey animals face a predator that have the above two characteristics, and predict a predation avoidance mechanism that has the following five presumption: 1) immobility of a prey animal induces slower approach of the predator that has already detected the prey, 2) the slow approach increases latency of the predator to attack, which incidentally makes the predator inconspicuous, 3) the increased latency and the inconspicuousness induce some other prey animals that do not recognize the existence of predator to come into the perceptual area of the predator before the predator initiates attack on the immobile prey, 4) the predator is distracted from the immobile prey to the new prey, and 5) the immobile prey survives as a result of the distraction. Similar distraction has been demonstrated in insects that exhibit death-feigning for increasing probability of survival at the expense of more mobile neighbors (Miyatake et al. 2009). Here, I examined feasibility of this mechanism by a staged-experiment using frogs as prey and snakes as predator.

I first examined the response of frogs against snakes slowly approaching them so as to confirm whether frogs exhibit immobility after being detected by snakes. Then, I examined whether snakes are distracted from an immobile frog to a moving frog during the slow approach to the immobile frog. Finally, to evaluate the feasibility of the occurrence of this phenomenon in nature, I measured the abundance of frogs simultaneously observed on a small area in a paddy field.

2-2 Methods

Study organisms

The subjects were *Pelophylax nigromaculatus*, and *Elaphe quadrivirgata*. A total of 48 frogs and 12 snakes were used for the experiments. The sampling sites, housing condition and treatment after the experiments were same as Chapter 1 except that frogs were housed in each terrarium by eight frogs.

Definition of predatory behavior in snakes

I defined the following four responses of *E. quadrivirgata* to frogs. Phase 1: Orienting – a sudden displacement of the head, head and neck, or the anterior part of the body in the direction of the prey. The position of the whole body does not change. Phase 2: Slow approaching – slow locomotion straight toward the prey. Phase 3: Pursuing – Rapid locomotion straight toward the prey. Phase 4: Striking – opening the jaws and projecting the head, head and neck, or the anterior part of the body rapidly toward the prey.

Experimental apparatus

The test arena, measuring 1175 × 452 × 425 mm, was made of clear glass panels. A box, measuring 400 × 300 × 300 mm, was put on the arena. The box was made of cardboards and had a window, which is able to be opened by

pulling up with a string. Two rings were attached at the corner of the arena, and strings that were tiled around the belly of each frog (see below) were passed through the rings (Fig. 4). All trials were filmed with a video camera (JVC GZ-HM1) by means of a mirror.

2-2-1 Experiment 1: examining response of frogs against an approaching snake, and measuring the increased latency

I introduced a snake inside the box and a frog outside the box in the arena. Distance between the frog and the box was at least 800 mm. Ten minutes after introducing the frog, I opened the window of the box. When the snake protruded its anterior part of the body from the box, I initiated a session and induced movement of the frog so that the snake detects the frog. To induce the frog to move, a string was tied around the belly of the frog. By pulling the string from outside of the arena, I induced the frog to perform locomotive movement similar to voluntary jumping. For this manipulation, I pulled the string basically once every five seconds. When the snake exhibited orienting, I immediately stopped pulling the string on seeing the orientation, and confirmed that the snake had not initiated pursuing. Three minutes later, I began recording behavior of the snake and frog with the aid of a video camera. If the snake did not exhibit orienting for 10 minutes after the initiation of pulling the frog, I abandoned the session. In an Experimental session, I recorded their interaction without pulling the string, and measured time until the snake struck the frog after the initiation of recording. In a Control session, I resumed pulling the string at the same time as the initiation of

recording, and measured time until the snake struck the frog after initiation of recording. If the snake struck the frog or moved other directions continuously for three minutes, I finished the session. I conducted 11 and eight trials in Experimental and Control session, respectively. Frogs and snakes were not used more than once in each session, but six snakes were used in both sessions. Nineteen frogs and 13 snakes were used. Mean body mass of the frogs was 5.8 g (range 2.1-25.9 g). Mean body mass and snout-vent length of the snakes was 232.3 g (range 77.5-455.0 g) and 986 mm (range 825-1180 mm), respectively.

2-2-2 Experiment 2: examination of distraction from an immobile frog to a moving frog

Experiment 2 consisted of Experimental sessions 1and 2 and control session. In Experimental session 1, I focused on whether a snake is distracted by a moving frog during the slow approach toward an immobile frog. In case that the snake was distracted, I continued to session 2 to examine the probability of survival of the immobile frog. Consequently, I conducted 12 session 1 and 11 session 2. In Control session, I examined probability of survival of the immobile frog without any other frogs. I conducted 12 Control sessions. Twenty-four frogs and 12 snakes were used throughout Experimental sessions 1 and 2. Twelve frogs and snakes each were used in Control session. No frogs were used more than once, but snakes were used repeatedly throughout Experimental sessions 1, and 2 and Control session. Mean body mass of the frogs was 4.4 g (range 1.6-11.5 g).

Mean body mass and snout-vent length of the snakes were 243.3 g (range 77.5-455.0) and 941 mm (range 737-1180 mm), respectively.

Experimental session 1

I introduced a snake inside the box and two frogs (frogs-A and B) outside the box in the arena. Distance between each frog and the box was at least 800 mm, between two frogs was at least 350 mm. Ten minutes after introducing the snake and frogs, I opened the window of the box. After the snake protruded its anterior part of body from the box, I induced movement of frog-A by pulling a string tied around its belly with the same way as Experiment 1. When the snake exhibited orienting, I stop pulling the string and began recording the behavior of the snake and frog with the aid of a video camera. If the snake did not exhibit orienting behavior for 10 minutes after the initiation of pulling the frog, I abandoned the session. When the snake approached the frog-A and reached 400 - 250 mm distance from it, I began pulling a string tied around frog-B and observed which frog (frog-A or frog-B) was struck by the snake. In case that frog-B was struck by the snake, I regarded frog-A as survived, and continued to session 2. In case that frog-A was struck by the snake, I regarded frog-A as dead and terminated the session (Fig. 5).

Experimental session 2

I continued observing behavior of frog-A while the snake was swallowing frog-B. After the snake finished swallowing and began locomotive movement, I examined whether frog-A is able to avoid predation of the snake. Three minutes

after the initiation of locomotion of the snake, I induced movement of frog-A with the string for three minutes. I defined the first three minutes as Before frog-A movement Session (BS) and the next three minutes as After frog-A movement Session (AS). I observed whether the snake resume approaching frog-A in each session. In case that frog-A was not approached by the snake in BS, I regarded frog-A as survived. In case that frog-A was approached by the snake in BS, I regarded frog-A as dead (Fig. 5).

Control session

I examined probability of survival of frog-A in the absence of any other frog. I conducted the same procedure as Experimental session 1 except that frog-B was not introduced. I introduced a snake inside the box and a frog (frog-A) outside the box in the arena. Distance between the frog and the box was at least 800 mm. Ten minutes after introducing the snake and the frog, I opened the window of the box. After the snake protruded its anterior part of body from the box, I induced movement of frog-A by pulling a string tied around its belly with the same way as in Experiment 1. When the snake exhibited orienting, I stop pulling the string and began recording behavior of the snake and frog with the aid of a video camera. If the snake did not exhibit orienting for 10 minutes after the initiation of pulling the frog, I abandoned the session. I defined survival of frog-A when the snake changed its locomotive direction from frog-A and did not resume direct approach for three minutes. I also defined dead of frog-A when the snake struck frog-A (Fig. 5).

2-2-3 Field Observations

To evaluate the feasibility of the occurrence of this phenomenon in nature, I surveyed the number of moving *P. nigromaculatus* within 10 m × 1 m area where *P. nigromaculatus* distributes densely, which was the path of paddy field in Kyoto Prefecture, Japan. Prior to survey, I confirmed the occurrence of predation events between *P. nigromaculatus* and *E. quadrivirgata* in this study site. I walked along the path of the paddy field and counted the number of frogs. The duration of each survey was approximately 30 sec. I conducted the survey once a month from April to November in 2013.

2-3 Results

2-3-1 Experiment 1

In Experimental session, frogs initially exhibited immobility. The snake approached directly with a series of loops on its entire body, which is a typical preparatory posture for strike (Kardong and Bels 1998), and finally struck and captured the frog. In three of the 11 trials, the frog fled by jumping away when the snake reached the vicinity of the frog, then the snake pursued and struck it. In the remaining eight trials, the snake struck the frog before it initiated flight. Mean duration from the initiation of recording to the initiation of the strike was 1715.8 sec (range: 126.1-6821.3). In Control session, the snake initiated

pursuing the moving frogs and captured them in all nine trials. Mean duration from the initiation of recording to initiation of the strike was 3.1 sec (range: 1.1-8.0). I conducted a statistical analysis for six Experimental and Control sessions, in which six snakes were used in each session. The duration from the initiation of recording to the initiation of the strike in Experimental session was significantly longer than that in Control session (Wilcoxon signed-rank test: P = 0.00216).

2-3-2 Experiment 2

In Experimental session 1, after frog-B moved, the snakes changed their locomotive direction and struck frog-B in 11 of the 12 trials. In the other trial, the snake kept approaching frog-A and struck it. The snakes significantly more frequently targeted frog-B than frog-A (Binomial test: P = 0.0063). In Experimental session 2, while the snake was swallowing frog-B, frog-A remained motionless in all trials. After swallowing, the snake initiated crawling but not toward frog-A in all 11 BS. On the other hand, the snakes resumed approaching frog-A and captured it in 10 of the 11 AS.

In Control session, frog-A survived in one of the 12 trials. In the other 11 trials, the snake reached directly the frog and captured it. The probability of survival of frog-A with another frog was significantly higher than that without any other frog (McNemar's test: $P < 0.005$; Table 2).

2-3-3 Field observations

The number of frogs increased from April to August, and then decreased to November (Fig. 6). The estimated density of frogs in the place was 0.2 frogs/m^2 (Apr), 1.0 frogs/m^2 (May), 1.7 frogs/m^2 (Jun), 3.3 frogs/m^2 (Jul), 3.6 frogs/m^2 (Aug), 2.1 frogs/m^2 (Sep), 0.9 frogs/m^2 (Oct), 0.0 frogs/m^2 (Nov).

2-4 Discussion

The results of Experiment 1 support the first and second presumptions: frogs exhibited immobility, and snakes approached slowly with a pre-strike posture against the immobile frogs. The results of Experimental session 2 demonstrated that the snakes do not resume approaching the immobile frog after eating the newly emerged frog even though they still have enough motivation to feed on additional frogs, supporting the forth and fifth presumptions. Regarding the third presumption, *P. nigromaculatus* is known to be densely distributed around paddy fields (Shinoda 1984), and the result of the field observation, especially from May to October, confirmed the densely destribution. This period overlaps with the active season of *E. quadrivirgata* (Fukada 1960). Thus, the presence of some other frogs in the vicinity of the event that a snake is approaching an immobile frog would not be an unrealistic assumption. In Chapter 1, it was demonstrated that the frogs exhibit immobility against a moving snake, but the frogs move at approximately 3 times per minute against a static snake. The slow approach of snakes toward the immobile frog is relatively static state, in which other frogs would not aware the existence of the snakes. Thus, it is likely that

some moving frogs that do not recognize the existence of predator come into a perceptual area of a snake during the slow approach toward an immobile frog. Therefore, the proposed predation avoidance mechanism is empirically demonstrated in, at least, a frog-prey and snake-predator system, and immobility after being detected by predator can be an adaptive choice to avoid predation.

The above mechanism is assumed a situation of one predator versus multiple prey. However, the increased latency of predator to attack may contribute to predation avoidance even in a situation of one predator versus one prey. Considering the food chain, animals that are predators of some prey animals are also preyed upon by other predators. Optimal theories regarding foraging efficiency and predation risk assume that longer foraging time expose animals to their predator for longer time, consequently increases predation risk (Bednekoff 2007: Quinn et al. 2012). Thus, the longer latency of predator to attack may increase the probability that the predator is preyed upon by other predators during the latency. In case of snakes, snake may be preyed upon by birds before reaching the immobile frogs because the path of paddy field is exposed to avian predators. Therefore, the increased latency may have a function of predation avoidance even if no other frogs present near the predation event, and thus, immobility under being detected by predator can be exhibited regardless the existence of other prey animals.

When confronted with a predator, prey is often in close proximity to conspecifics (Hamilton 1971). This situation has generated several hypotheses regarding anti-predator strategies adopted by individuals within groups of

gregarious species, such as collective detection that all members of the group are unambiguously alerted to presence of predator as long as the predator is detected by at least one group member (Lima 1995). Moreover, even in short-term temporary aggregations of non-gregarious animals, the collective detection has been confirmed, and it does not necessarily need alarm call (Martin et al. 2006; Fernández-Juricic et al. 2009; Pays et al. 2013). For example, frogs are known to change their anti-predator responses according to the occurrence of escape behaviors of other individuals (Martin et al. 2006). However, for some prey animals that attempt surviving at expense of conspecific individuals, the system of collective detection should reduce the probability of successful expense of other individuals because it allows them to exhibit defensive behaviors (Hamilton 1971). On the other hand, frogs in the present study exhibited immobility on seeing snakes, which would not alert conspecifics to the occurrence of the predator. In addition, because the snake became relatively static in response to the immobile frog, it would become difficult for other frog individuals to recognize the presence of the snakes. Thus, exhibiting immobility on seeing predator incidentally hides the occurrence of predator from conspecifics, and increases probability of successful expense of them.

Chapter 3: Decision making after being detected by a predator snake, part 2

" Waiting for strike of predator snakes for successful escape in frogs "

3-1 Introduction

Broom and Ruxton (2005) model predicted that the optimal decision for cryptic prey is either fleeing immediately on seeing the predator, or not initiating fleeing until the predator has detected the prey. Thus, under being detected by predator, the prey has no choice but to initiate fleeing immediately. This, prediction is based on two presumptions: 1) immobility functions only to enhance crypsis and thus, it becomes no longer effective under being detected by predator 2) probability of successful escape decreases as the flight initiation is delayed because the delay allows predator come close. However, some prey animals do not initiate fleeing immediately even when predator has detected them (Brodie et al. 1974; Lagos et al. 2014). In Chapter 2, I demonstrated that immobility is still effective to avoid predation even under being detected by predator. In addition to the effectiveness of immobility after the detection by predator, non-immediate escape under detection have some advantages for avoid predation. In this chapter, I explored factors of successful escape by focusing on kinematic features of escape behavior of frogs and strike of snakes.

Escape of frogs relies on jumping (Toledo et al. 2011), which takes frogs in mid-air for a short distance. Because of the absence of friction to the ground, most of frogs except for gliding frogs (McCay 2001) are rarely able to change

their direction of escape in the air. On the other hand, capture of locomotive prey by snakes is achieved by crawling up to the prey and lunging the remaining distance (Cundall and Greene 2000). At the end of the lunging, snakes open their jaws and project the head, head and neck, or the anterior part of the body rapidly toward the prey, which is basic definition of snake strike (Cundall and Greene 2000). Basically in strike, a third or less of the trunk is recruited to move the head, with the remainder of the trunk remaining essentially stationary, for serving inertia as the launching platform for the head (Cundall and Greene 2000). Thus, it is suggested that once snakes initiate strike, they are rarely change their direction of strike. Moreover, the strike requires preparatory postures that consist of curve(s) on its bodies to create rapid forward movement by straightening the curve(s) (Kardong and Bels 1998). This implies that once snakes fail to capture prey by strike, they are not able to strike again until they make curve(s) at their trunks. Overall, it is likely that frogs and snakes hardly change their motion for a while after initiating jumping escape and strike, respectively. Thus, it is suggested that the timing of flight initiation of frogs relative to initiation of snake strike would be important factor to determine the outcome of the predation event between them.

In the present study, I first measured duration and area of the predation event between a frog and a snake based on field observation to confirm natural condition at predation events. Then, I examined the sequence of their interaction and kinematically measured their escape and predatory actions that would determine the outcome of predation event. Finally, based on the results of the experiments, I propose several important factors that would affect the decision of flight initiation of frogs.

3-2 Methods

Study organisms

The subjects were *Pelophylax nigromaculatus*, and *Elaphe quadrivirgata*. A total of 50 frogs and 23 snakes were used for the experiments. The subjects, sampling sites, housing condition and treatment after the experiments were same as Chapter 1 except that frogs were housed in each terrarium by eight frogs.

Experimental apparatus

The floor of test arena 1, measuring 2 × 2 m, was made of steel wire net covered with white cloth. The arena was enclosed its three sides by walls (2 m) made of plasterboard, and the other side by blue cloth (Fig. 7a). A hole was opened on the center of the floor, of which diameter was 5 mm, and the string was passed through the hole toward underside of the floor and tied around the belly of a frog on the floor. The floor of the arena was lifted up to 70 cm from floor of the room with pedestals so that I am able to observe their shadows from underside of the arena and manipulate frogs by pulling the string. A box, measuring 400 × 300 × 300 mm, was put on a corner of the arena. The box was made of cardboards and had a window that I am able to open by pulling up with a string (Fig. 7c). All trials were filmed from side by a high-speed video camera (KEYENCE VW-9000, 1000 frames per seconds) and top by a video camera (JVC GZ-HM1, 60 frames

per second).

The test arena 2, measuring 1175 × 452 × 425 mm, was made of clear glass panels. The box same as that of test arena 1 was put on the corner of test arena 2 (Fig. 7b). A ring was attached on top of the glass panel of the arena, and the string was passed through the ring and tiled around the belly of a frog for controlling its movements. (Fig. 7b). All trials were filmed from side and top by means of a mirror with a video camera (JVC GZ-HM1, 60 frames per second).

Definition of predatory behavior of snakes

I defined the following four responses of *E. quadrivirgata* to frogs. Phase 1: Orienting – a sudden displacement of the head, head and neck, or the anterior part of the body in the direction of the prey. The position of the whole body does not change. Phase 2: Slow approaching – slow locomotion straight toward the prey. Phase 3: Chasing – Rapid locomotion straight toward the fleeing prey. Phase 4: Striking – opening the jaws and projecting the head, head and neck, or the anterior part of the body rapidly toward the prey.

3-2-1 Field observations

To confirm natural condition at predation events, I measured duration and area of the predation event between a frog and a snake by field observation. The observations were made on 6 June 2012, 27 June 2013 and 1 July 2013 at paths of small paddy field surrounded by deciduous broad-leaved and evergreen coniferous forests in Iwakura and Shizuhara, Sakyo, Kyoto Prefecture, Japan. I

walked along the path, looking for foraging snakes. When I found *E. quadrivirgata*, I stalked it to observe predation event between the snake and a frog. The predation events were directly observed and were recorded with a video camera (JVC GZ-HM1, 60 frames per second). All durations (s) are obtained as result of dividing the number of the frames by 60.

3-2-2 Experiment 1: observation of the sequence of predation event

To examine the sequence of interaction between a frog and a snake during escape of frogs, I conducted a staged encounter experiment on a floor of which size was similar to the horizontal size of predation event at the field observations. I introduced a snake inside the box and a frog outside the box on the center of the test arena 1 (Fig. 7a). By pulling the string from underside of the arena, I was able to hold the position of the frog near the center of the arena and also induce the frog to perform locomotive movement similar to voluntary jumping. Distance between the frog and the box was approximately 1 m. Ten minutes after introducing the frog and the snake, I opened the window of the box. When the snake protruded its anterior part of the body from the box, I induced movement of the frog by pulling the string from outside of the arena basically once every five seconds so that the snake detects the frog. When the snake reached approximately 40cm distance from the frog, I cut the string as short as possible at underside of arena so that the frog is able to move freely. Then, I initiated the session and began recording behavior of the frog and snake with the aid of a video camera (JVC GZ-HM1, 60 frames per second) from upper side. I finished the session when either the snake captured the frog, the snake returned to the

box, head of the snake reached the edge of the arena or the frog reached the edge of the arena. I conducted 10 trials, and 10 frogs and eight snakes were used. Frogs were not used more than once, but two snakes were used repeatedly. Mean body mass of the frogs was 5.2 g (range 2.8-7.2 g). Mean body mass and snout-vent length of the snakes was 243 g (range 76-455.0 g) and 942 mm (range 695-1130 mm), respectively.

3-2-3 Experiment 2: measurement of durations of behavioral acts

I introduced a snake inside the box and a frog outside the box in the test arena 2. Distance between the frog and the box was at least 800 mm. Ten minutes after introducing the frog, I opened the window of the box. When the snake protruded its anterior part of the body from the box, I initiated a session and induced movement of the frog so that the snake detects the frog. By pulling the string from outside of the arena basically once every five seconds, I induced the frog to perform locomotive movement similar to voluntary jumping. When the snake exhibited orienting, I cut the string so that the frog is able to move freely, and then I began recording behavior of the snake and frog with the aid of a video camera (JVC GZ-HM1, 60 frames per second). If the snake did not exhibit orienting for 10 minutes after the initiation of pulling the frog, I abandoned the session. If the snake captured the frog or moved other directions continuously for three minutes, I finished the session.

To estimate an area that snakes are able to capture frogs during a strike, I first defined the following two lines. One is a line of sight from the snake to the

frog at the initiation of strike, and the other is a line from head of the snake at initiation of strike to a point at which the trajectory of the snake head in strike intersected which the trajectory of the frog in flight when the frog successfully evaded the strike (Fig. 8). I measured an angle of these two lines (hereafter I refer it as the maximum adjustable angle of strike, Fig. 8) by video analysis of snakes and frogs.

For detail analysis of interaction between frogs and snakes, I defined the following moments of behavioral acts of snakes and frogs. Strike initiation: when snakes initiate launching their head from preparatory posture of strike. Strike end: when the anterior trunk of snakes is completely straightened after strike. Chase initiation: when snakes initiate locomotive movement rapidly toward the fleeing frog but do not strike. Flight initiation: when frogs initiate locomotive movement for fleeing. Take off: when all of their body parts of frogs separated from the floor of the arena. Touch down: when frogs touch any part of their body to the floor after the preceding Take off.

To determine factors that affect outcome of chase of frogs by snakes, I measured the following durations. All durations (s) are obtained as result of dividing the number of the video frames by 60. In case that the chase began by strike movement of the snake on the stationary frog, I measured (1) duration from Strike initiation to Flight initiation (Response-f), (2) duration from Flight initiation to Take off (Kicking), (3) duration from the first Take off to the first Touch down (Air 1), (4) duration from the first Touch down to the second Take off (Ground 1), (5) duration from the second Take off to the second Touch down (Air 2), (6) duration from the second Touch down to the third Take off (Ground 2),

(7) duration from Flight initiation to Chase initiation (Flight to Chase), (8) duration from Strike initiation to Strike end (Strike1), (9) locomotive velocity of head of the snake from Strike initiation to Strike end, (10) duration from Strike end to Chase initiation (Post-Strike). In case that the chase began by fleeing of the frog prior to strike, I measured (2), (3), (4), (5), (6), (7) and (11) distance between a frog and a snake at Flight initiation (Flight initiation distance). Flight-Chase is referred to as response time of snakes to a fleeing frog (Response-s) in this case for the convenience of description of figure (see Results). I excluded behavioral acts that are apparently disturbed by a string or clear panels. I conducted 40 trials. Frogs were not used more than once. Twenty-three snakes were used, of which nine snakes were used more than once. Mean body mass of the frogs was 6.6 g (range 1.0-25.9 g). Mean body mass and snout-vent length of the snakes was 233 g (range 46-455 g) and 939 mm (range 501-1180 mm), respectively.

3-3 Results

3-3-1 Field observations

Four cases of behavioral interaction between a snake and a frog were observed. The observed interactions occurred on fair days in June and July at air temperature of 27.3-28.4 °C.

Observation 1: I noticed a snake crawling along a path of the paddy field and

began recording it at 13 h 45 min on 6 June 2012. Air temperature was 27.5 °C. A frog initiated fleeing and the snake chased it with strike. Consequently strike of the snake failed and the frog survived by one jump toward the water (Table 3). The distance between the position of the snake at the initiation of strike and that at the end of chasing was 1 m. The duration between the initiation of jumping of the frog and the end of chasing by the snake was 1.250 sec.

Observation 2: I continued observing the snake after the observation 1. The snake kept crawling along with the path of the paddy field. Air temperature was 27.6 °C.A frog initiated fleeing and the snake chased it. Consequently, the frog survived by one jump toward the water (Table 4). The distance between the position of the snake at the initiation of chasing and that at the end of chasing was 0.5 m. The duration between the initiation of jumping of the frog and the end of chasing by the snake was 1.067 sec.

Observation 3: I noticed a snake crawling on a steep upsloping bank at 4 m outside of paddy field and began recording it at 15 h 11 m on 27 June 2013. Air temperature was 27.3 °C. A frog initiated fleeing and the snake chased it. Consequently, the frog survived by three jumps toward grass (Table 5). The distance between the position of the snake at initiation of strike and that at the end of chasing was 3 m. The duration between the initiation of jumping of the frog and the end of chasing by the snake was 1.43 sec.

Observation 4: I noticed a snake crawling along a path of the paddy field and began recording it at 1613 h on 1 July 2013. Air temperature was 28.4 °C. Consequently, the frog survived by one jump toward the ditch (Table 6). The distance between the position of the snake at the initiation of strike and that at

the end of chasing was 1 m. The duration between the initiation of jumping of the frog and the end of chasing by the snake was 1.317 sec.

3-3-2 Experiment 1

After cutting the string tied around the belly of the frog, the frog initially exhibited immobile state in all 10 trials. The snake approached directly and slowly with a series of loops on its entire body, which is a typical preparatory posture for strike (Kardong and Bels 1998), in all trials. Subsequently to the direct and slow approach, a chase of the frog by the snake was observed in seven of the 10 trials. The shift from the slow approach to the chase was triggered by flight movement of frogs in three trials and by strike of snakes in four trials. The frog eventually reached the edge of the arena without being captured in all seven trials. In the other three trials, the snake initiated striking the frog, and the frog did not exhibit fleeing in response to the strike and was captured.

3-3-3 Experiment 2

I confirmed that 27 trials contained a chase: 11 chases were triggered by strike and 16 chases were triggered by fleeing of frogs. In the other 13 trials, the snake did not strike the frog or the frog did not flee. In the 11 strike-triggered chases, six events that the frog evaded the forestall strike were suitable for estimating the area that snakes are able to capture frogs during a strike. Mean of the

maximum adjustable angle of strike was 4.1 ° (range: 0-11 °). The sequence of their behaviors on the strike-triggered chase was 1) the snake initiated strike, 2) the frog evaded the strike by jumping and fled by continuous jumping, and 3) the snake initiated chase. Mean ± SD of each duration were mentioned on Table 7 (see Fig. 9). On the other hand, the sequence of their behaviors on the flight-triggered chase was 1) the frog initiated jumping and fled by continuous jumping, and 2) the snake initiated chase. Mean ± SD of velocity of the snake head from Strike initiation to Strike end was 93 ± 34 cm/s. Mean ± SD of each duration were mentioned on Table 7 (see Fig. 9). Flight-Chase at strike-triggered chase was significantly longer than that at flight-triggered chase (Welch's test: $P < 0.05$, Fig. 9).

3-4 Discussion

The result of field observations showed that water zones and grass around the predation event work as temporary refuges for frogs against snakes. Thus, accessing these refuges by a few meter movements, which is carried out by a few times of jumping, results in successful escape for frogs. The results of Experiment 1 indicated that frogs are inevitable to be chased by snakes while moving to refuges, and the chase is triggered by either fleeing of frogs or strike of snakes. The results of Experiment 2 demonstrated that snakes hardly change their trajectories of strike. Most of the strikes recruit anterior part of the trunk to move the head, with the remainder of the trunk remaining essentially stationary

to serve inertia for launching the head (Cundall and Greene 2000). Thus, snake strike is accelerated straightly along with a line from stationary part of the posterior trunk to the head. This mechanism should unable snakes to change direction while striking. Thus, it is likely that frogs are able to evade the strike with a certain distance at the initiation of strike. This minimum distance for evasion (D) can be estimated by velocity of strike (V) and time for frogs to evade the strike (T) as following formula.

$$D = V \times T$$

V is obtained by the mean locomotive velocity of the snake head from Strike initiation to Strike end, which is 93 cm/sec. When snakes strike the frog, they would aim at the position of the frogs on the surface of the ground. To evade the straightly and horizontally launched head, a little vertical locomotion would be sufficient for the frogs. Hence, I assumed that frogs successfully evade strike by achievement of their take off. Thus, T equals to the duration from Strike initiation to Take off, which consists of Response-f and Kicking. I used mean Response-f and Kicking, which is 0.159 sec and 0.050 sec, respectively. Therefore,

$$D = 93 \text{ cm/sec} \times (0.159 \text{ sec} + 0.050 \text{ sec})$$

$$= 19 \text{ cm}$$

Within this distance, approximately 19 cm, frogs would not be able to evade forestall strike, and they should initiate fleeing before initiation of strike. This may be a reason that frogs initiated fleeing at 21.9 cm distance on average in Experiment 2. On the other hand, at more than the distance, frogs would be able to evade strike even after it is launched. Therefore, frogs may be able to select the type of chase initiation, flight-triggered chase by forestall fleeing or

strike-triggered chase by waiting for initiation of snake strike.

The probability of reaching refuge of frogs may change according to the type of chase initiation. The results of Experiment 2 indicate that snakes are not able to begin the subsequent predatory acts immediately after exhibiting strike behavior. When performing strike, snakes produce strong thrust power by straightening curve(s) of their trunks (Cundall and Greene 2000). Thus, as the result of straightening, they lose potential to produce additional thrust. This must be the reason for the occurrence of a short lag between the end of strike and the initiation of the subsequent chase. Hence, once frogs evade the strike of snakes, they consequently acquire a split second to flee without being chased by the snakes. Considering the short time taken to access refuges in the field observations, even the momentary pause that is caused by evading the first strike would be sufficient to increase a chance for frogs to reach the refuge.

In addition to the pause after strike, flight initiation after strike initiation causes predator to come close and pass the vicinity of the prey when the strike is evaded by the frog. This effect has two advantages of successful escape. First, it enhances prey to be positioned out of predator sight. Predatory animals usually have their two eyes positioned on the front of their heads, thereby allowing for binocular vision and reducing their field of view in favor of stereopsis (Platel R 1994; O'Rourske et al. 2010; Larsson 2013). As mentioned in Discussion of Chapter 1, fleeing at the shorter distance causes greater changes in the angle between the longitudinal axis of the head of the snake and the line from the snake to the frog. Moreover, the angle would increase rapidly and greatly when predator is passing the vicinity of the prey. Consequently, predator

may lose sight of the prey and not be able to initiate chasing appropriately (see Howland 1974). Second, it removes predator from the expected escape course of prey. In nature, it occurs that predator positions between the prey and the refuge. In the field observation, snakes crawled along the path of paddy field, and frogs at outer side of the path than the snakes must be obstructed by the snakes to access the paddy field directly. In such situations, evading the strike of the predator replaces the predator from a position between prey and refuge to an external position between them. Thus, fleeing after initiation of strike enables the prey to escape directly toward a refuge without obstruction of predator.

On the other hand, flight before the initiation of strike does not have the above possible advantages and also may increase the risk of being captured because of kinematic characteristics of fleeing of frogs. At jumping of frogs, acceleration and direction changes are produced by kicking force to the ground, then they travel in mid-air where barely no reduction of their velocity and no change of trajectory are possible because of no friction to the ground. Thus, frogs in fleeing should be either in mid-air with rapid locomotion of constant velocity and parabolic trajectory or on the ground with slow locomotion. Hence, flight initiation prior to snake strike allows snakes to adjust the direction of strike to the moving frog in mid-air. Indeed, at the Observation 1, the frog initiated fleeing and then the strike of the snake passed the close vicinity of the frog. While the strike passed, the frog did not change its trajectory. Moreover, it may allow snakes to strike the frogs at landing, in which the frogs are not able to move quickly. Therefore, flight initiation of frogs prior to the initiation of snake strike may decrease the probability of successful escape.

In conclusion, flight initiation of frogs after initiation of snake strike may increase the probability of successful escape, and thus, frogs may wait for strike of snakes until they closely approach can be an adaptive decision that results in head start. This mechanism may explain the response of frogs that they do not initiate fleeing even when they have been detected by snakes. In Broom and Ruxton (2005) model, the probability of successful escape is assumed to decrease with shortening the distance between predator and prey, and they predicted initiation of fleeing is simply triggered by detecting predator or being detected by predator. However, the present study suggests that considering the detailed behavioral acts of predator and prey, of which kinematic characteristics must be varied according to species, is important to determine the optimal response of predation avoidance in the real world.

GENERAL DISCUSSION

In Chapter 1, it was demonstrated that frogs initially exhibit immobility against a remote predator, and they switch from immobility to fleeing at a timing between detecting predator and being detected by predator, which is obvious discordance with the prediction of the Broom and Ruxton (2005) model. To explain this discordance, I proposed two new viewpoints for understanding interplay between predator and prey: 1) engagement of intensive searching mode by predator at short distance, which leads it to eventually detect the prey, and 2) close-quarters effect that produces an additional defensive function on fleeing only within a close proximately of predators. In Chapter 2, frogs remain motionless even when snakes have detected the frogs, which is in discordance with current interpretation of function of immobile state against predator. I demonstrated that immobile state has function to increase latency of predator to attack, and it results in distracting the predator to other prey. In Chapter 3, it was suggested that flight initiation of frogs after the initiation of snake strike may increase the probability of successful escape because snakes hardly change their strike trajectory after initiating strike and are unable to move immediately after the end of striking. Collectively, it was suggested that the decision of switching from immobility to fleeing does not necessarily depend on detection of predator, the trigger of switching is likely to be the distance between the frog and predator snake and/or the occurrence of strike.

The anti-predator mechanisms proposed in each chapter can be applied not only on between *P. nigromaculatus* and *E. quadrivirgata*, but also on other prey

and predator systems. The decision making of frogs in Chapter 1 may be adaptive for some prey animals that use immobility for crypsis against predator that actively searches prey with multiple cues, for example mice against weasels (King and Powell 2006). The decision making of frogs in Chapter 2 may be adaptive for some prey animals living with other conspecifics against visually hunting predator in stalking, for example crickets against jumping spiders, (Jackson and Pollard 1996; Miyatake et al. 2009; Lagos et al. 2014). The decision making of frogs in Chapter 3 may be adaptive for some prey animals of which successful escape depends on timing of initiation of escape, for example fishes against predator fishes and snakes (Catania 2009; Stewart et al. 2013).

Immobile state has been considered as a representative defensive behavior among many animals. It is generally thought that immobile state can be classified into two types: tonic immobility and the others according to whether it is spontaneous or not, and the latter is often called immobility (Herzog 1984; Greene 1988). Tonic immobility is a state of natural paralysis and it has been considered to be exhibited mainly against predator of approaching and subjugating phase (Endler 1991; Miyatake et al, 2004, 2008, 2009; Ruxton et al. 2004; Gerald 2008). Its function has been considered as at least death-feigning, physical defense, loss of predator's interest and signaling of unpalatability (Miyatake et al. 2009). On the other hand, the immobility is a voluntarily immobile state. It has been considered to be exhibited against predator in searching phase (Endler 1991), and its function has been considered only as enhancing crypsis (Endler 1991, Ruxton et al. 2004). Thus, this classification has been useful because each immobile state corresponds to predatory phase of predator: tonic immobility, which is performed against predator in approaching

and subjugating phases, and immobility, which is performed against predator in searching phase. However, in the present study, immobility was demonstrated to be also performed against predator in approaching phase (Chapter 2). Thus, the classification according to spontaneousness would not be suitable to divide immobile state in correspondence with predatory phase of predator. In addition, terminologically immobility can cover tonic immobility. To avoid confusion of terminology of immobile state, I propose a definition to describe immobile state of prey animals. First, "immobility" covers every immobile state. Then, according to spontaneousness, immobility is classified into "tonic immobility" and "spontaneous immobility" (Mori 1991), but the classification is independent from correspondence with predatory phase.

Broom and Ruxton (2005) model predicted flight initiation is triggered by detection of prey or predator. However, my study demonstrated that there are other factors that affect the timing of flight initiation: intensive searching mode of predator, close-quarters effect, latency of predator to attack, presence of another nearby prey and kinematic features of predatory behavior and escape behavior. Incorporating these factors into theoretical models will be a fruitful challenge to determine the optimal response of prey for predation avoidance. Although the present study was conducted in a simplified environmental condition, and examinations in more natural setting would be required, I anticipate that new findings in the present study will contribute to better understanding of the anti-predator strategy of animals in the real world.

ACKNOWLEDGEMENTS

I am most grateful to Associate Professor Akira Mori for his advice and criticism throughout this study. Many thanks are due to Professor Hideharu Numata for his invaluable comments and suggestions on the study. Dr. Elizabeth Nakajima provided helpful comments on various versions of manuscripts and kindly corrected the English expressions. I am indebted to Dr. Daisuke Muramatsu, Dr. Teppei Jono and Kohei Okamoto for helping with the statistics. Takashi Ishikawa and Kouji Kishimoto are thanked for helping with building a computer program for data analysis. I also thank Dr. Naoyuki Fuse and Keita Tsujimoto for allowing me to use their computer software for analyzing C-trax data, Professor Alan H. Savitzky, Dr. Hirohiko Takeuchi, Dr. Yohei Kadota and Dr. Yosuke Kojima for helpful comments, Fumiaki Mizugami, Tomohiro Matsuzawa, Sora Shimonaka and Ai Obuchi for their assistance in sampling *P. nigromaculatus* and *E. quadrivirgata*, Takuya Yokoyama and Tomoha Matsuo for constructing experimental devices, Anzu Ikeda, Daisuke Takahashi, Eri Hayashi, Kazutaka Hosoda, Keiko Tsubokawa, Masahiro Uesaka, May Hayashida, Naoki Yamamoto, Nobuhiko Hamazaki, Rio Tsutsumi, Shiori Fujinami, Shota Nakanoh, Dr. Sylwia Hyniewska, Takashi Kawase, Tokie Sakai, Yoshichika Taira, Yoshika Matsui, Yuko Urata and Yuri Nishikawa for various helps and encouragements, and many local people in Iwakura and Shizuhara for permitting to use their own paddy fields for field observations and sampling materials.

This work was partially supported by the Global COE Program A06, Grants for Biodiversity & Evolution Project of Excellent Graduate Schools to Kyoto

University from MEXT, Japan and the Sasakawa Research Grant (26-528) to Nozomi Nishiumi from the Japan Science Society.

I conducted all experiments at a laboratory in Kyoto University in compliance with the guidelines of the Animal Care and Use Committee of Kyoto University.

REFERENCES

Bednekoff PA (2007) Foraging in the face of danger. In: Stephens DW, Brown JS, Ydenberg RC (eds) Foraging. University of Chicago Press, Chicago, pp 305-320

Brodie ED, Johnson JA, Dodd CK (1974) Immobility as a defensive behavior in salamanders. Herpetologica 30:79-85

Brodie ED, Formanowicz Jr DR, Brodie III ED (1991) Predator avoidance and antipredator mechanisms: distinct pathways to survival. Ethol Ecol Evol 3:73-77

Broom M, Ruxton GD (2005) You can run - or you can hide: optimal strategies for cryptic prey against pursuit predators. Behav Ecol 16:534-540

Burger J (1974) Breeding adaptations of Franklin's gull (*larus pipixcan*) to a marsh habitat. Anim Behav 22:521-567

Caro T (2005) Antipredator defenses in birds and mammals. University of Chicago Press, Chicago

Mattison C (1995) The encyclopedia of snakes. Facts on File, New York.

Catania KC (2009) Tentacled snakes turn C-starts to their advantage and predict future prey behaviour. Proc Natl Acad Sci USA 106:11183-11187

Cooper WE (1997) Factors affecting risk and cost of escape by the Broad-Headed Skink (*Eumeces laticeps*): predator speed, directness of approach, and female presence. Herpetologica 53:464-474

Cooper WE (2003) Effect of risk on aspects of escape behavior by a lizard, *Holbrookia propinqua*, in relation to optimal escape theory. Ethology 109:617-626

Cooper WE Frederick WG (2007) Optimal flight initiation distance. J Theor Biol 244:59-67

Cooper WE Pérez-Mellado V, Baird TA, Baird TD, Caldwell JP, Vitt LJ (2003) Effects of risk, cost, and their interaction on optimal escape by nonrefuging Bonaire whiptail lizards, *Cnemido- phorus murinus*. Behav Ecol 14:288-293

Cundall D, Greene HW (2000) Feeding in snakes. In: Schwenk K (ed) Feeding: Form, Function, and Evolution in Tetrapod Vertebrates. Academic Press, San Diego, pp 293-333

Dawkins R, Krebs JR (1979) Arms races between and within species. Proc R Soc Lond B 205:489-511

Ducey PK, Brodie Jr ED (1983) Salamanders respond selectively to contacts with snakes: survival advantage of alternative antipredator strategies. Copeia 1983:1036-1041

Duellman WD, Trueb L (1994) Biology of Amphibians. McGraw-Hill, New York

Edmunds M (1974) Defence in animals: a survey of anti-predator defences. Longman, London

Endler JA (1991) Interactions between predators and prey. In: Krebs JR, Davies NB (eds) Behavioural Ecology: An Evolutionary Approach. Blackwell Scientific, Oxford, pp 169-196

Fernández-Juricic E, Delgado JA, Remacha C, Jiménez MD, Garcia V, Hori K (2009) Can a solitary avian species use collective detection? An assay in semi-natural conditions. Behav Proc 82:67-74

Ford JKB, Reeves RR (2008) Fight or flight: antipredator strategies of baleen whales. Mammal Rev 38:50-86

Fukada H (1960) Biological studies on the snakes. VII. Growth and maturity of *Elaphe quadrivirgata* (Boie). Bull Kyoto Gakugei Univ B 16:6-21

Gamberale-Stille G, Bragée C, Tullberg BS (2009) Higher survival of aposematic prey in close encounters with predators: an experimental study of detection distance. Anim Behav 78:111-116

Gerald GW (2008) Feign versus flight: influences of temperature, body size and locomotor abilities on death feigning in neonate snakes. Anim Behav 75:647-654

Goris RC, Maeda N (2004) Guide to the Amphibians and Reptiles of Japan. Krieger Publishing, Florida

Greene HW (1988) Antipredator mechanisms in reptiles. In: Gans C, Huey RB (eds) Biology of the Reptilia. Vol. 16. Alan R Liss, New York, pp 1-152

Halpern M (1987) The organization and function of the vomeronasal system. Annu Rev Neurosci 10:325-362

Hamilton WD (1971) Geometry for the selfish herd. J Theor Biol 31:295-311

Herzog HA (1984) Tail autotomy inhibits tonic immobility in geckos. Copeia 1984:763-764

Howland HC (1974) Optimal strategies for predator avoidance: the relative importance of speed and manoeuvrability. J Theor Biol, 47:333-350

Jackson RR, Pollard SD (1996) Predatory behavior of jumping spiders. Annu Rev Entomol 41:287-308

Kadowaki S (1996) Ecology of a Japanese snake community - resource use patterns of the three sympatric snakes, *Rhabdophis tigrinus*, *Elaphe quadrivirgata* and *Agkistrodon b. blomhoffii*. Bull Tsukuba Univ Forests 12:77-148 (in Japanese)

Kardong KV, Bels VL (1998) Rattlesnake strike behavior: kinematics. J Exp Biol 201:837-850

King MC, Powell RA (2006) The natural history of weasels and stoats: ecology, behavior, and management. Oxford University Press, Oxford

Lagos A, Ebensperger LA, Herberstein ME (2014) A quantitative test of the 'economic' and 'optimal' models of escape behaviour. Anim Behav 97:221-227

Larsson M (2013) The optic chiasm: a turning point in the evolution of eye/hand coordination. Front Zool 10:41

Lima SL (1995) Collective detection of predatory attack by social foragers: fraught with ambiguity? Anim Behav 50:1097-1108

Maeda N, Matsui M (1999) Frogs and Toads of Japan (Revised ed). Bun-ichi Sogo Shuppan, Tokyo (in Japanese)

Marchisin A, Anderson JD (1978) Strategies employed by frogs and toads (Amphibian, Anura) to avoid predation by snakes (Reptilian, Serpentes). J Herpetol 12:151-155

Martin J, Luque-Larena JJ, Lopez P (2006) Collective detection in escape responses of temporary groups of Iberian Green Frogs. Behav Ecol 17:222-226

McCay MG (2001) Aerodynamic stability and maneuverability of the gliding frog *Polypedates dennysi*. J Exp Biol 204:2817-26

Miyatake T, Katayama K, Takeda Y, Nakashima A, Sugita A, Mizumoto M (2004) Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour. Proc R Soc B 271:2293-2296

Miyatake T, Tabuchi K, Sasaki K, Okada K, Katayama K, Moriya S (2008) Pleiotropic antipredator strategies, fleeing and feigning death, correlated with dopamine levels in *Tribolium castaneum*. Anim Behav 75:113-121

Miyatake T, Nakayama S, Nishi Y, Nakajima S (2009) Tonically immobilized selfish prey can survive by sacrificing others. Proc R Soc B 276:2763-2767

Mori A (1991) Spontaneous immobility of the Japanese lacertid lizard, *Takydromus tachydromoides.* Jpn J Herpetol 14:1-5

Mori A, Moriguchi H (1988) Food habits of the snakes in Japan: a critical review. Snake 20:98-113

O'Rourke CT, Hall MI, Pitlik T, Fernández-Juricic E (2010) Hawk eyes. I. Diurnal raptors differ in visual fields and degree of eye movement. PLoS ONE 5:e12802

Ota H (1986) Snake really an able hunter?: predatory behavior of Japanese striped snake, *Elaphe quadrivirgata*, in the field. J Ethol 4:69-71

Pays O, Beauchamp G, Carter AJ, Goldizen AW (2013) Foraging in groups allows collective predator detection in a mammal species without alarm calls. Behav Ecol 24:1229-1236

Platel R (1994) Nervous system and sensory organs. In: Bauchot R (ed) Snakes: A Natural History. Sterling, New York, pp 50-59

Quinn JL, Cole EF, Bates J, Payne RW, Cresswell W (2012) Personality predicts individual responsiveness to the risks of starvation and predation. Proc R Soc B 279:1919-1926

Ruxton GD, Sherratt TN, Speed MP (2004) Avoiding attack: the evolutionary ecology of crypsis, warning signals, and mimicry. Oxford University Press, Oxford

Shinoda N (1984) Frogs of the *Rana nigromaculata* group from iwate prefecture: their distribution and variation in external characters. Jpn J Herpetol 10:97-103 (in Japanese)

Shinohara N (2007) The effect of the new-style of rice paddies on the distribution in *Rana nigromaculata*, Kagawa Prefecture, Japan. Kagawa Seibutsu 34:97-105 (in Japanese)

Siegel S, Castellan NJ (1988) Non-parametric statistics for the behavioral sciences (2nd ed). McGraw-Hill, New York

Stewart WJ, Cardenas GS, McHenry MJ (2013) Zebrafish larvae evade predators by sensing water flow. J Exp Biol 216:388-398

Toledo LF, Silva RR, Haddad CFB (2006) Anurans as prey: an exploratory analysis and size relationships between predators and their prey. J Zool 271:170-177

Toledo LF, Sazima I, Haddad CFB (2011) Behavioural defences of anurans: an overview. Ethol Ecol Evol 23:1-25

Vallin A, Jakobsson S, Lind J, Wiklund C (2005) Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits. Proc R Soc B 272:1203-1207

Van Valkenburgh B (1985) Locomotor diversity within past and present guilds of large predatory mammals. Paleobiology 11:406-428

Wasson K, Lyon BE (2005) Flight or fight: flexible antipredatory strategies in porcelain crabs. Behav Ecol 16:1037-1041

Wattiez R, Remy C, Falmagne P, Toubeau G (1994) Purification and preliminary characterization of a frog-derived proteinaceous chemoattractant eliciting

prey attack by checkered garter snakes (*Thamnophis marcianus*). J Chem Ecol 20:1143-1160

Ydenberg RC, Dill LM (1986) The economics of fleeing from predators. Adv Stud Behav 16:229-249

Zhang H, Yan J, Zhang G, Zhou K (2008) Phylogeography and demographic history of Chinese black-spotted frog populations (*Pelophylax nigromaculata*): evidence for independent refugia expansion and secondary contact. Evol Biol 8:21-36

Table 1 The number of snakes that detected frogs in Experiment 2, Chapter 1

Experiment	Behavior of frogs	
	Motionless	Moving
Long distance (400-800 mm)	0 (20)	7 (7)
Short distance (0-100 mm)	8 (9)	14 (20)

Numerals in parentheses are the total number of trials.

Table 2　Analysis using MacNemar's test on the results of survival of frog-A against 12 snakes in Experimental sessions (With a moving frog) and Control sessions (without any other frog). Numerals are number of trials.

		Without any other moving frog	
		Survived (n=1)	Dead (n=11)
With a moving frog	Survived (n=11)	1	10
	Died (n=1)	0	1

Table 3 Time sequence of frog-snake interaction at observation 1, Chapter 3.

Time	Behavioral description
13 h 55 min 49.800 s	A frog initiated jumping at 3 cm distance from the snake toward inside of the paddy field.
13 h 55 min 49.867 s	The snake initiated striking the fleeing frog in mid-air at 7 cm distance from the snake.
13 h 55 min 49.967 s	The snake failed to hit the frog.
13 h 55 min 50.067 s	The snake oriented and initiated chasing the frog still in mid-air at 1 cm distance from the snake.
13 h 55 min 50.150 s	The frog reached water surface of the paddy field, and then dived into the water.
13 h 55 min 50.267 s	The snake reached the water surface where the frog dived.
13 h 55 min 51.050 s	The snake stopped chasing the frog.
13 h 55 min 53.817 s	The snake resumed crawling along the path of the paddy field.

Table 4 Time sequence of frog-snake interaction at observation 2, Chapter 3.

Time	Behavioral description
14 h 35 min 7.483 s	A frog on the path at 2 cm distance from the snake initiated jumping toward inside of the paddy field.
14 h 35 min 7.567 s	The snake initiated chasing the fleeing frog in mid-air at 3 cm distance from the snake.
14 h 35 min 7.683 s	The frog reached the water surface of the paddy field, and then dived into the water.
14 h 35 min 7.750 s	The snake reached the water surface where the frog dived.
14 h 35 min 8.550 s,	The snake stopped chasing the frog.
14 h 36 min 49.833 s	The snake resumed crawling along the path of the paddy field.

Table 5　Time sequence of frog-snake interaction at observation 3, Chapter 3.

Time	Behavioral description
15 h 34 min 1.133 s	A frog jumped from the outside of the paddy field toward the paddy field. Although I could not see the frog and head of the snake at the initiation of the jump because of grass covered them, the distance between the frog and the snake was estimated as approximately 10 cm.
15 h 34 min 1.283 s	The snake initiated chasing the fleeing frog in mid-air at 30 cm distance from the snake.
15 h 34 min 1.550 s	The frog went out of sight of the video camera, but it was directly observed: the frog jumped twice and then disappeared into grass.
15 h 34 min 2.567 s	The snake stopped chasing the frog.
15 h 34 min 15.083 s	The frog initiated jumping at 50 cm distance from the snake.
15 h 34 min 15.267 s	The snake initiated raising its head.
15 h 34 min 15.750 s	The snake kept its posture with raising its head 30 cm above the ground.
15 h 34 min 29.083 s	The snake initiated lowering its head and then resumed crawling.

Table 6 Time sequence of frog-snake interaction at observation 4, Chapter 3.

Time	Behavioral description
16 h 24 min 34.017 s	A frog on the path at 5 cm distance from the snake initiated jumping toward an irrigation ditch filled with water along the path.
16 h 24 min 34.050 s	The snake initiated chasing the jumping frog at 6 cm distance from the snake.
16 h 24 min 34.567 s	The frog reached water surface of the ditch and dived into the water.
16 h 24 min 34.983 s	The snake reached the water surface where the frog dived.
16 h 24 min 35.333 s	The snake stopped chasing the frog.
16 h 24 min 46.600 s	The snake resumed crawling along the path.

Table 7　Durations of behavioral acts of a frog and a snake at predation event and flight initiation distance of frogs. See text for explanations of each duration.

Type of Chase	Variables		Mean	SD	N
Flight-triggered chase	Kicking	(sec)	0.073	0.047	14
	Air1	(sec)	0.209	0.129	13
	Ground1	(sec)	0.163	0.074	12
	Air2	(sec)	0.189	0.080	9
	Ground2	(sec)	0.119	0.031	9
	Flight-Chase (Response-s)	(sec)	0.111	0.054	14
	Flight initiation distance	(cm)	20.9	21.8	16
Strike-triggered chase	Response-f	(sec)	0.159	0.121	11
	Kicking	(sec)	0.050	0.022	11
	Air1	(sec)	0.257	0.043	7
	Ground1	(sec)	0.128	0.076	6
	Air2	(sec)	0.163	0.069	4
	Ground2	(sec)	0.158	0.058	2
	Strike1	(sec)	0.261	0.147	6
	Post-Strike	(sec)	0.206	0.235	6
	Flight-Chase	(sec)	0.436	0.320	7

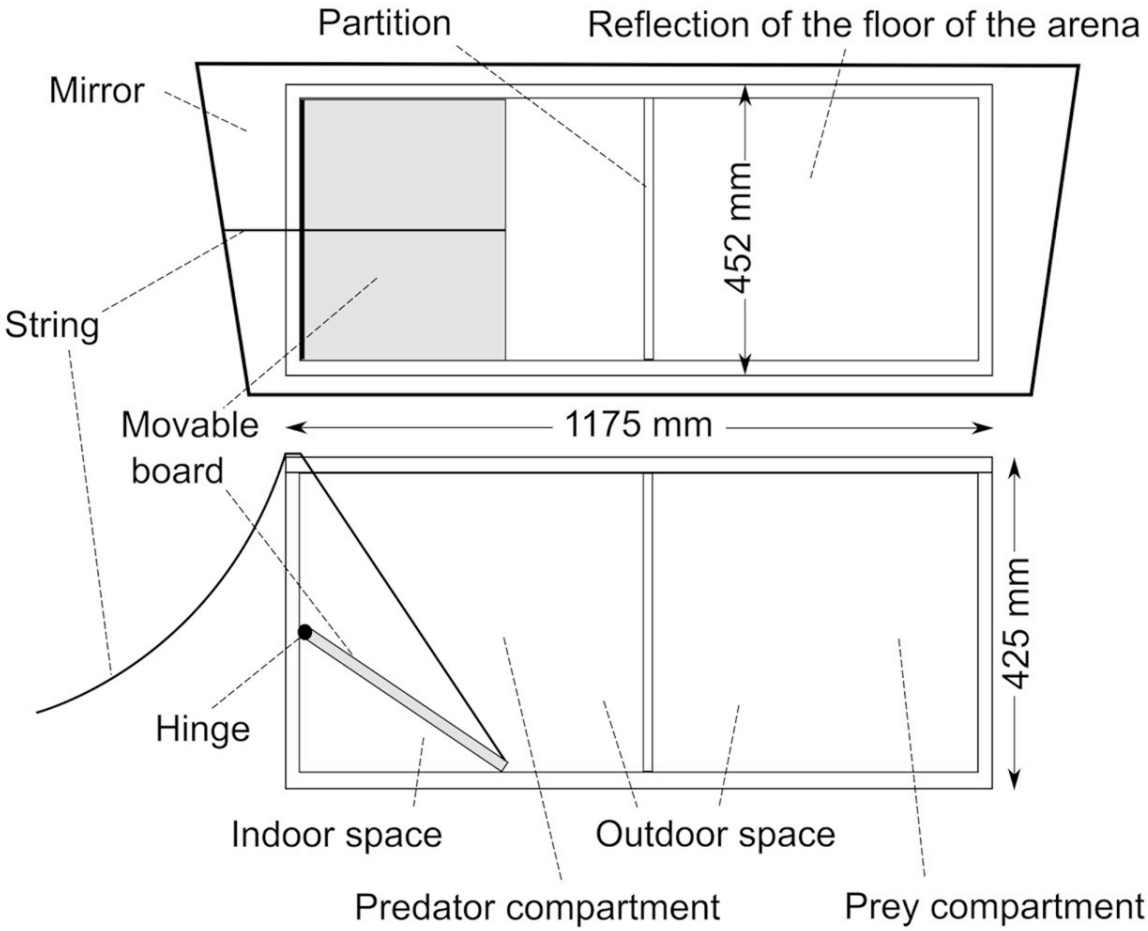

Fig. 1　Top (upper) and side (lower) views of the test arena used in the experiments in Chapter 1. See text for detailed descriptions.

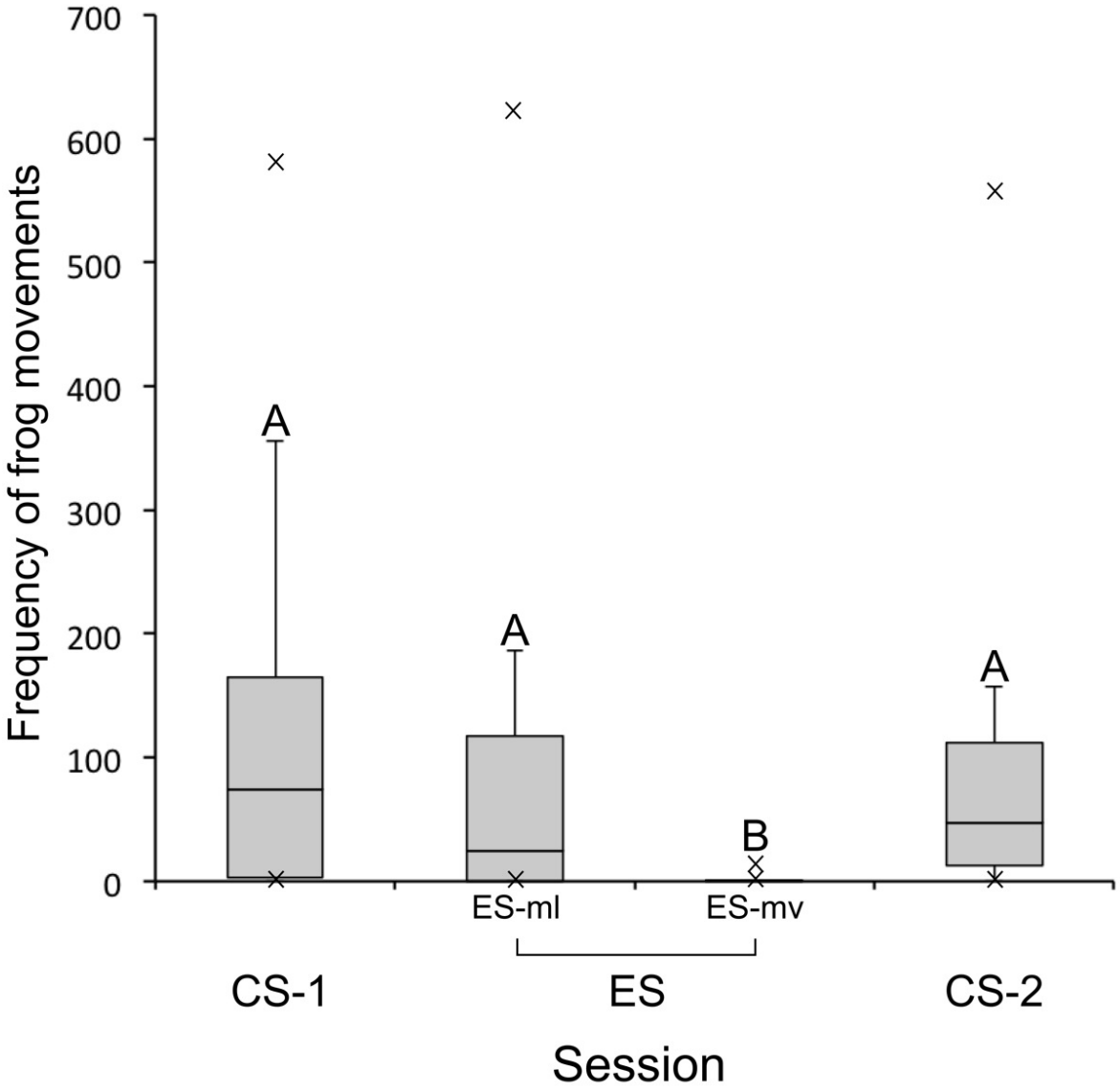

Fig. 2　Box plot of the rate of frog movements (the number of movements per hour). ES is an experimental session in which a frog movements are monitored in the presence of a snake. CS is a control session (no snake) conducted before (CS1) or after (CS2) the experimental session. ES-ml and ES-mv indicate the period when the snake remained motionless and when the snake was moving, respectively. Different letters above the boxes indicate significant differences (multiple comparison: $|R_u-R_v| \geq 20.43$, $P < 0.05$).

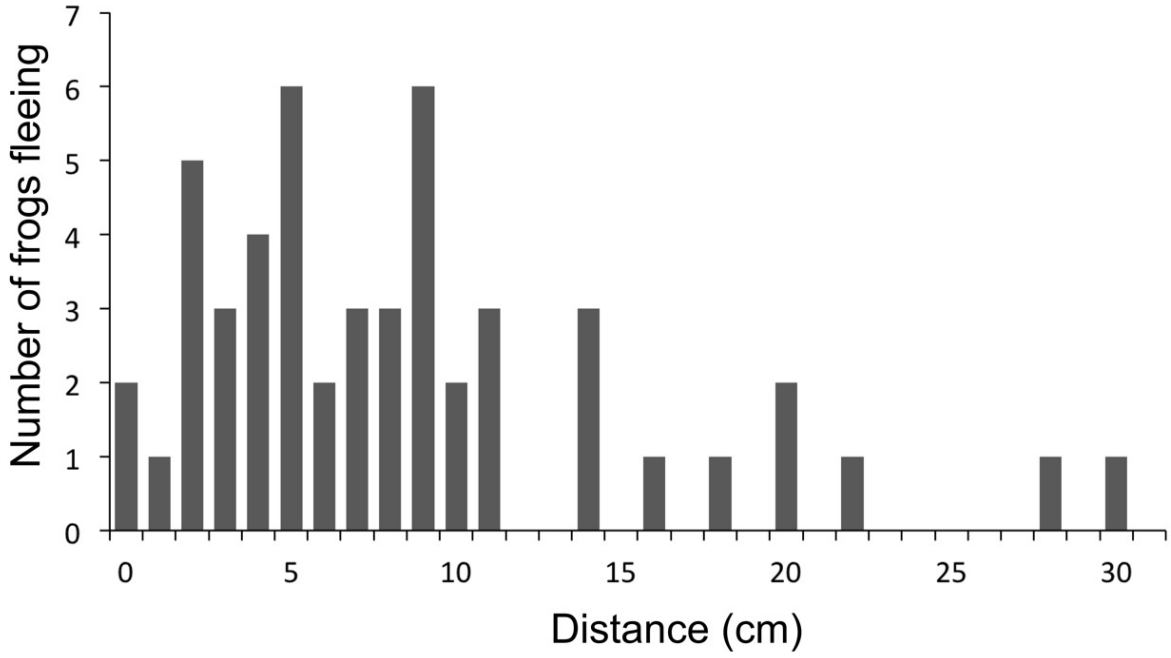

Fig. 3　Frequency distribution of the distance between a frog and a snake when the frog started fleeing in response to the approaching snake.

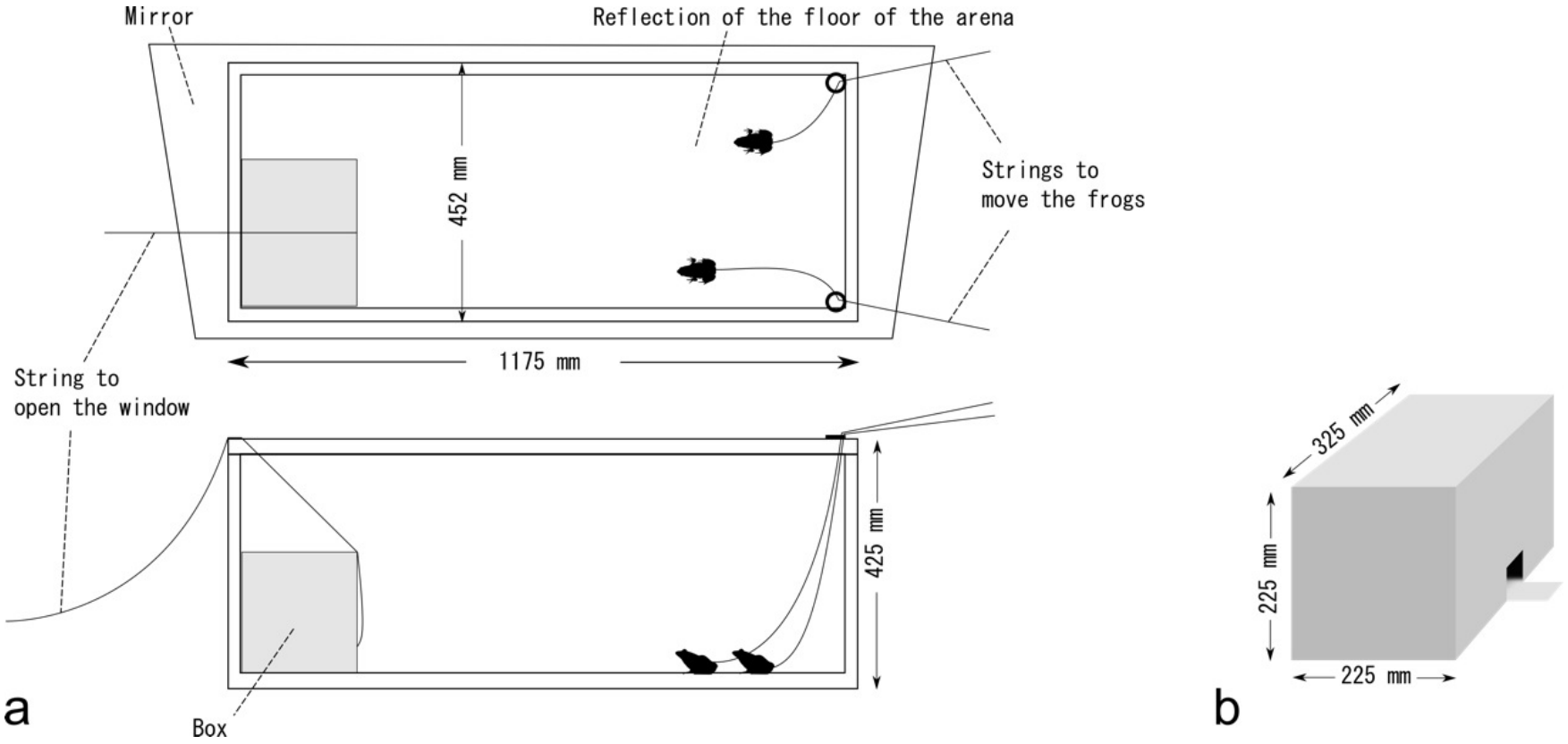

Fig. 4 (a) Top (upper) and side (lower) views of the test arena used in the experiments in Chapter 2. See text for detailed descriptions. (b) The exterior of the box.

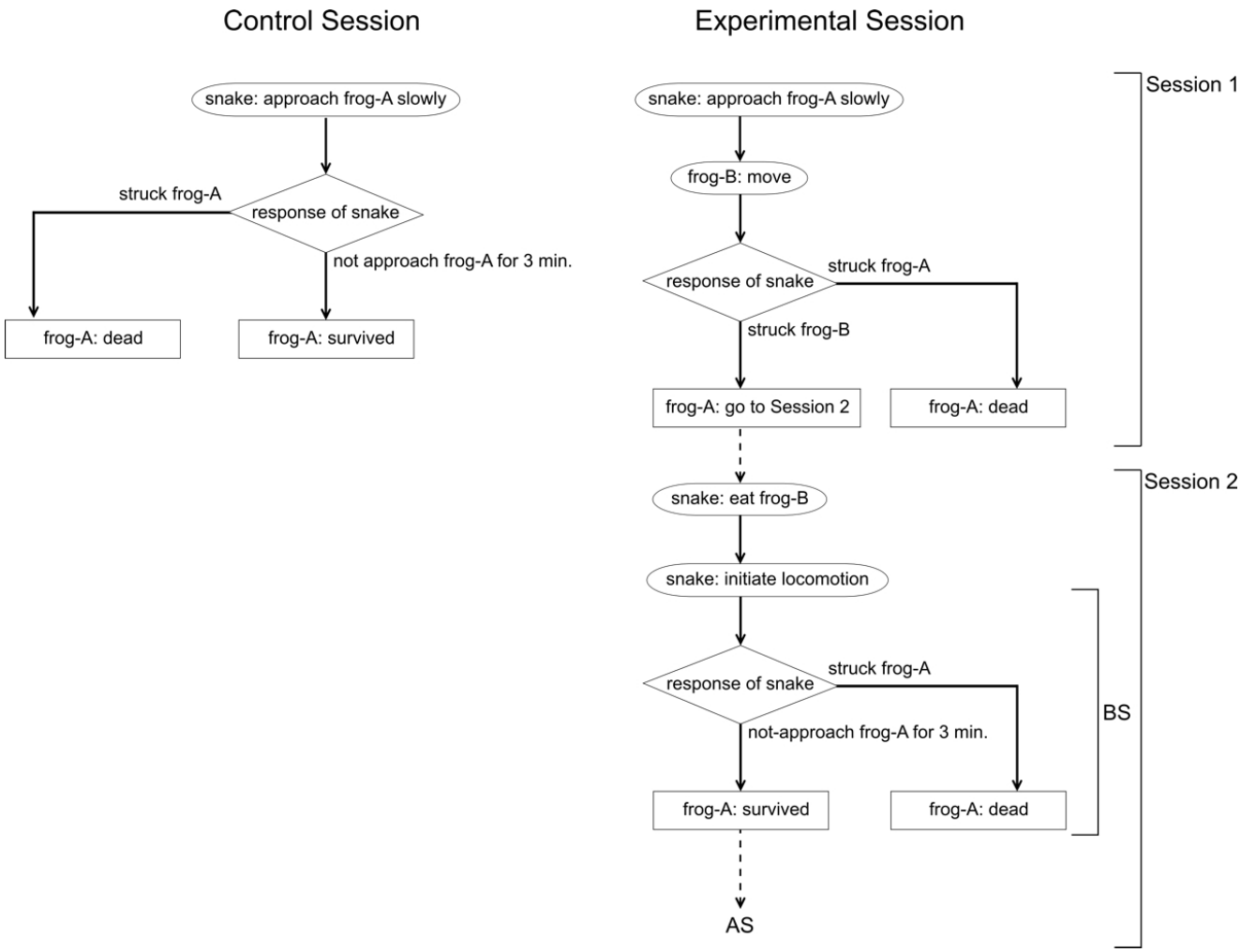

Fig. 5　Experimental flow of Experiment 2 in Chapter 2

The right side is a flow of Experimental session, in which two frogs were used against a snake. Below the part of Before movement Session (BS), the part of After movement Session (AS) was omitted. The left side is a flow of Control session, in which one frog was used against a snake.

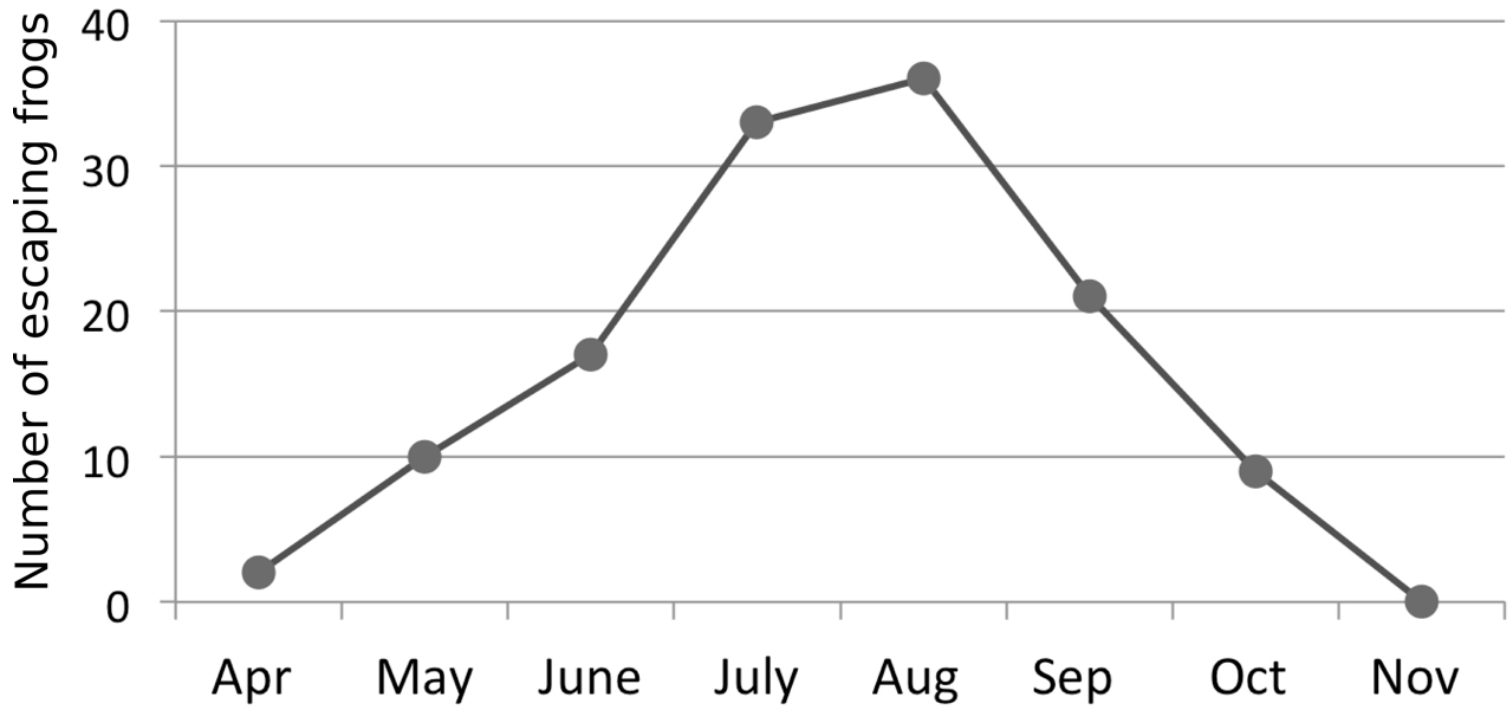

Fig. 6 Number of frogs observed within 10 m^2 path of paddy field in Kyoto, Japan from Apr to Nov in 2013.

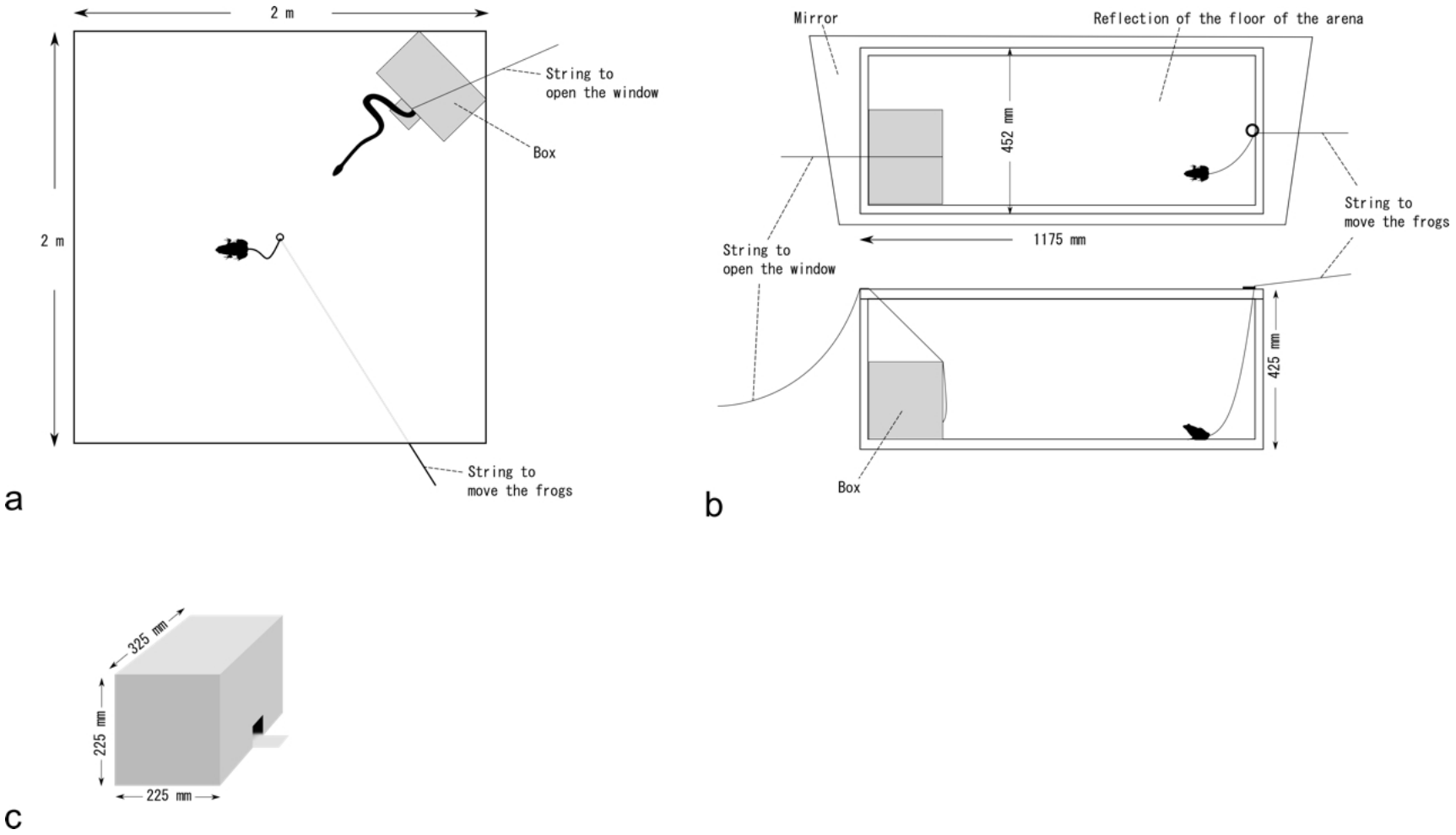

Fig. 7 (a) Top view of the test arena 1. (b) Top (upper) and side (lower) views of the test arena 2. See text for detailed descriptions. (c) The exterior of the box.

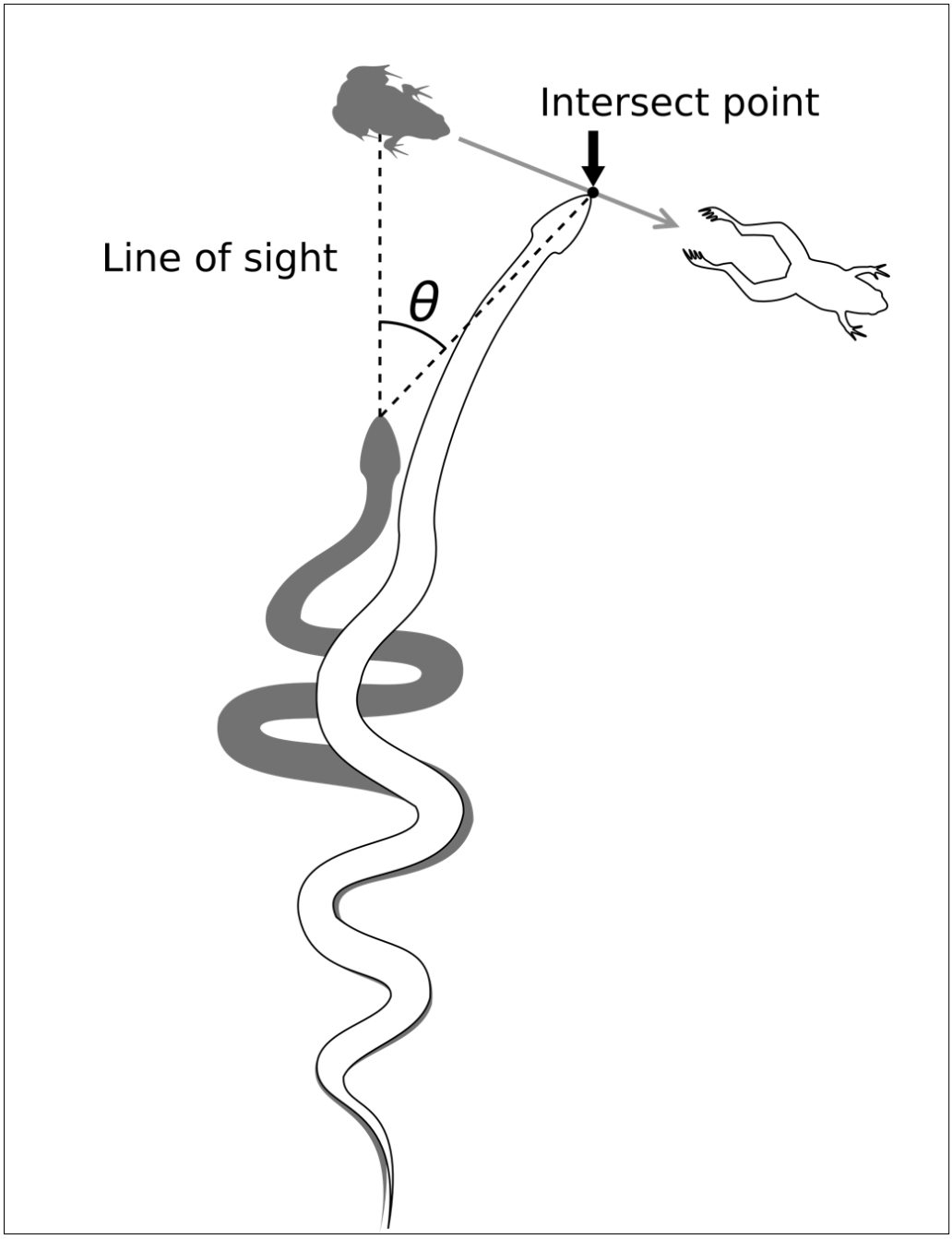

Fig. 8　Schematic view of the interaction of a frog and a snake to measure the maximum adjustable angle for strike (θ). Positions of the animals at the initiation of strike are indicated grey color. Positions of the animals at evasion of strike are indicated by white color. Line of sight is a line from the center of frog to the head of the snake at the initiation of strike. Intersect point is a point at which the trajectory of strike and that of fleeing frog are crossed.

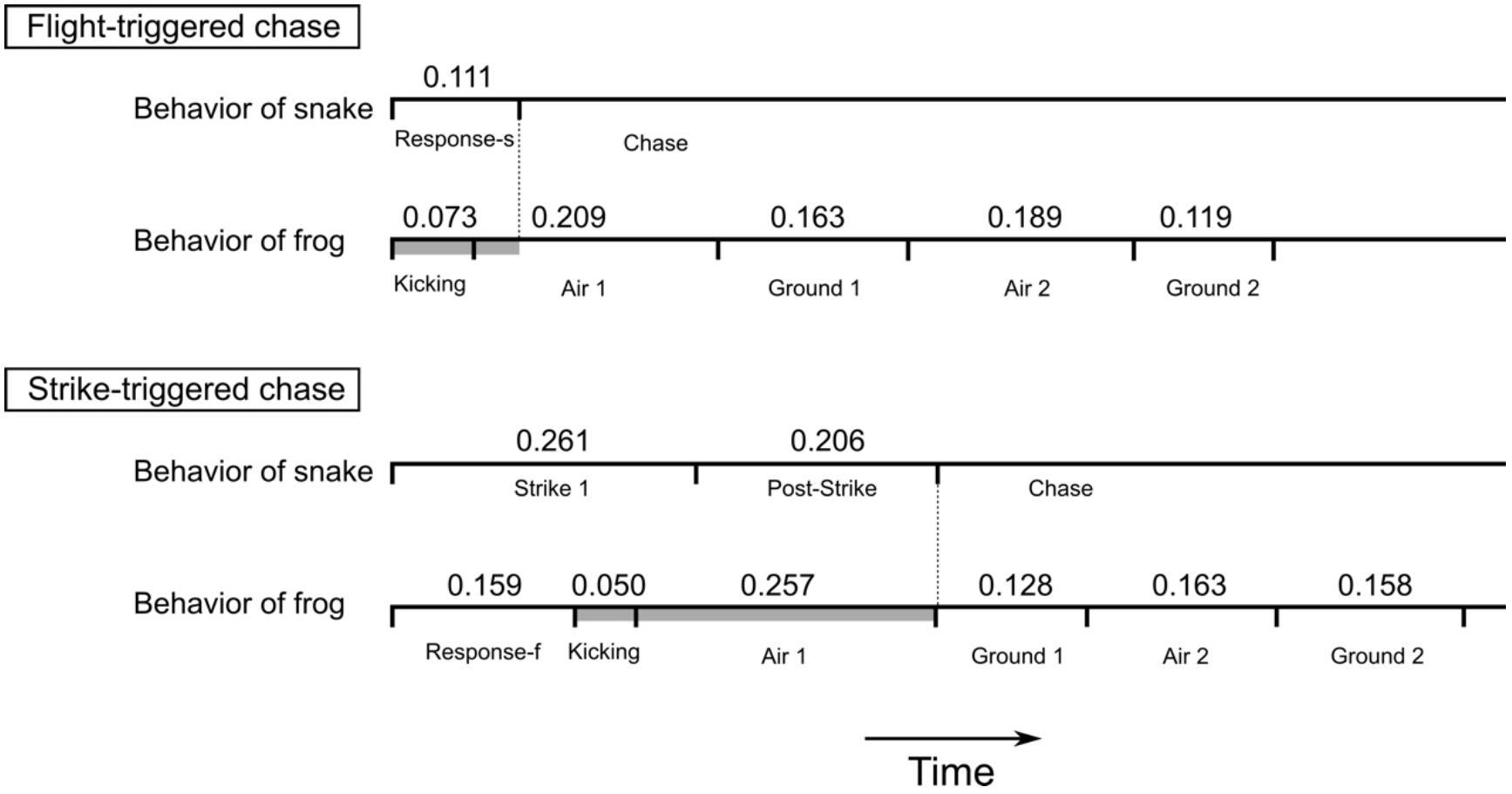

Fig. 9 Time sequence of interaction between a frog and a snake at the initiation of predatory chase. The numbers indicate mean duration of each behavior. Grey bars indicate Flight-Chase.