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Orientation of the Toad, *Bufo japonicus*, toward the Breeding Pond

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ABSTRACT—A variety of orientation cues has been suggested for the migration to the breeding site in adult amphibians. We categorized the cues into the following 3 groups: 1) cues from the breeding pond such as male calling and pond odors, 2) celestial cues such as the sun light and the magnetic field of the earth and 3) cues from the area or route of the migration which compose a local map such as a visual and olfactory maps. To determine which of these is used by the toad, *Bufo japonicus*, we designed and conducted a displacement experiment in which migrating toads from one direction were transported to the ground in the opposite side of the pond. The displaced toads were completely disoriented and moved to random directions. We conclude that the toad uses a local map to orient to the breeding pond and cues from celestial bodies and the pond are not used. We also found that adult toads tracked the same route on both trips from and to the pond. This suggests that the local map was memorized by newly metamorphosed toads at their first terrestrial trip from the pond. The next step of our study was to determine what sense is used to receive the cue. We found blind toads, whose upper and lower eye-lids were stitched together, could reach the pond at a similar rate with the sham-operated and intact toads. However, anosmic toads, whose olfactory mucosa were damaged by the treatment with a 5% silver nitrate solution, rarely reached the pond. Tracking experiments with blind toads and toads bearing a bar magnet on their heads revealed that neither blindness nor the magnet had any effect on either the direction or distance of the migration to the pond. In contrast, anosmic toads could not orient properly and moved in random directions. We speculate that newly metamorphosed toadlets remember the route or area of their first terrestrial migration by using the olfactory sense. In the spring, two to three years later, they start their breeding migration to the home pond by using a local olfactory map and reverse tracking of the memorized route.

INTRODUCTION

The orientation of amphibians to their breeding site or home range has been extensively studied and various cues have been shown to be used for the orientation (for review see [1, 17, 18]). Opinion is divided even in toads of Bufonidae: celestial in *Bufo woodhousei fowleri* [5], acoustic in *B. americanus* [15], magnetic in *B. bufo* [16], *B. spinulosus* [18] and *B. calamita* [19], olfactory in *B. bufo* [16] and visual cues in *B. calamita* [19] have been suggested to be used for the orientation. In *B. calamita*, it was reported that the type of the orientation cue used differed by gender: males used olfactory and magnetic cues and females solely an acoustic cue [16].

Adult toads of *B. japonicus*, as well as its close relative *B. bufo* in Europe, migrate to certain ponds for breeding in the spring. However, no systematic study has been published on orientation of *B. japonicus* to the breeding site. Since many types of cue have been suggested, even in amphibians of the same genus, we commenced our study on the orientation of *B. japonicus* without relying on previously published data. Firstly, we made the following three choices for possible origins of orientation cues: 1) celestial bodies, 2) pond for breeding and 3) local area or route of migration.

To determine which of these serves as the origin of the cue in *B. japonicus*, we designed and conducted a special displacement experiment. Secondly, we observed whether or not toads used the same route both moving toward and away from the breeding pond. If they use the same route on both trips, the route must have been used in their first terrestrial trip, i.e. postmetamorphic exodus from the home pond. Thirdly, we conducted a recapture experiment to ascertain what sense is used to obtain the cue for orientation to the breeding pond. We examined the visual and olfactory senses. Lastly, we studied effects of elimination of the visual or olfactory sense, as well as securing a magnet on the head, on the direction and distance of the migration to the breeding pond. From the results of these observations and experiments, we speculated how *B. japonicus* does orient to the breeding pond.

MATERIALS AND METHODS

Materials

Bufo japonicus, the common toad in Japan and once classified as a subspecies of *B. bufo*, is morphologically and ecologically similar to *B. Bufo* in Europe. According to Urano and Ishihara [23], adult toads of *B. japonicus* are nocturnal and actively prey on small animals through a period from May to November in Tokyo and its vicinity. In late November, as the ambient temperature falls, they burrow into the ground or under litter and become torpid. They remain there until early spring. On humid and relatively warm nights between

the middle of February and late March, they appear on the surface of the ground and migrate toward certain breeding sites, mostly ponds. Adult males visit the same pond every year but females every other or third year at least in Tokyo.

The earliest and latest dates of breeding of *B. japonicus* for the last 15 years in Tokyo were 14th February and 29th March, respectively (Ishii, unpublished). Males are more numerous than females at the breeding sites, while the sex ratio is about 1 : 1 in the summer [9]. In the breeding season, amplexus is frequently observed in the pond and less frequently on the ground near the pond. Soon after breeding, they return to the ground and stay torpid again under the ground until May or June, when the ambient temperature becomes high enough for the predatory activity. Thus, their winter torpid period is interrupted by breeding activity.

The present study was done only in the breeding season with adult males and females of *B. japonicus* found on the ground in several places in and near Tokyo. After experiments were completed, all toads used were released at the places where they were captured.

Designs of observations and experiments

Preliminary field observation: We intended to confirm that migrating toads maintain their direction toward a certain breeding pond and do not move randomly. We also intended to ascertain their hours of migration. This observation was performed around a pond on the campus of a school (Jiyu Gakuen, Higashi-Kurume City, Tokyo) between 17th and 21st March, 1980.

Displacement experiment: A passive displacement experiment was designed to determine the origin of the orientation cue in this species. We classified possible orientation cues into three categories according to their origins: the first is a physical or chemical cue from the breeding pond, such as male calling, a chemical substance in pond water or humidity originated from water in the pond, the second is a celestial cue such as the magnetic field of the earth or the sun compass, and the third is a cue from the migration route or local area of migration creating a route or area map. To test which of these three types of cue is employed by *B. japonicus*, we conducted the following displacement experiment. We captured more than 60 toads moving toward a pond at locations about 50 m from the south-east shore of the pond. They were divided at random into two of the experimental and control groups of the same size. Each toad of the experimental group was put individually into a box soon after capture and carried to the shore at the opposite side of the pond. Then they were released at locations 50 m from the pond shore. Control toads were carried to the middle point between the locations of their capture and the release of the experimental toads, then turned around, returned to the original locations and released there. If experimental (displaced) toads move toward the pond, move in the opposite direction to the pond or get lost, the first, second or third categories of the cues, respectively, must be used by *B. japonicus*. The experiment was conducted in a pond area in the grounds of a temple (Heirin-Ji, Niiza City, Saitama Prefecture) between 21st and 27th March, 1983. Prior to this experiment, an observation of the migration angle in normal intact toads was performed between 12th and 19th March, 1982 at the same place.

Observation of the migration route from the pond: We divided a section (140 m long) of a road located 50–80 m to the west from the pond at Heirin-Ji into 4 different zones of the same length and toads migrating toward the pond across the road were marked with plastic tape in four different colors (red, white, green and blue), according to the zones they crossed. The length of each zone was 35 m.

Marking was performed between 15 and 18th March, 1983. At about 19 : 00 on 27th March, we monitored the color of toads moving away from the pond and crossing each zone of the road. Thus, we could determine whether or not toads used the same route on the both trips (to and from the pond).

Recapture experiment with blind toads and anosmic toads: Migrating male and female toads were captured in areas about 50 to 100 m from a pond in Koganei Park, Koganei City, Tokyo on 11th March 1987. Twenty of them were individually tagged (intact control). Twenty and 15 were blinded mechanically and sham-operated, respectively. Twenty-four and 17 were made anosmic chemically or sham-operated, respectively. All these treatments were done in the field soon after capture. As soon as the procedures were finished, all the toads were released at the same locations where they were captured. Many anosmic toads did not move through the night of the operation. Every evening from 12th to 17th March (except 16th March), we collected all toads found in the breeding pond and examined their marks. The recapture rate of the operated toads was determined for each operation group.

Tracking experiments with blind toads, anosmic toads and toads bearing a magnet: Experiments to study the effects of elimination of visual sense, olfactory sense or of a magnet on the direction and distance of migration were designed. The blind toad experiment was performed in the grounds of National Institute of Environmental Research, Tsukuba City, Ibaragi Prefecture between 10th and 25th March, 1987; the anosmic toad experiment at Koganei Park, Koganei City, Tokyo between 17th February and 1st March, 1988; and the magnet experiment on the main campus of Tokyo University of Agriculture and Engineering, Fuchu City, Tokyo between 10th and 18th March, 1995.

In the blind toad experiment, 30 migrating toads were captured. Twenty of them were immediately blinded mechanically or sham-operated; the remaining 10 were tagged only. They were released at the locations of their capture soon after the operations. Migration routes of the released toads were tracked individually through the night of the release. Seven blind, 7 sham-operated and 9 intact toads could be tracked successfully. In two out of the 7 blind toads, the operation was found to be incomplete, and accordingly they were excluded from data.

In the anosmic toad experiment, 75 migrating toads were captured on humid and warm nights (17th, 20th and 21st February, 1989), and the exact locations of capture were recorded individually. They were immediately put in a box kept chilled with ice. After being carried back to the laboratory, 25 of the toads were made anosmic and the same number sham-operated. The remaining 25 toads were left intact. After the operations, all toads of the three groups were kept at 5°C in a cold room. During the next humid and warm nights (28th February and 1st March 1989), they were released at the same locations of capture between 18 : 00 and 20 : 00 and their routes were tracked individually throughout the night of the release. Because of malfunctioning tracking devices, 4 and 2 toads were lost in the sham-operated and intact groups, respectively.

In the magnet experiment, 93 migrating toads were captured on humid and warm nights between 10th and 17th March, 1995 and were allocated to 4 different kinds of treatment with a random allocation table. For toads of the first and second (experimental) treatment groups, a bar magnet was glued on the head of each toad. In the first group, the south pole of the magnet was set upward (abbreviated as S-magnet group), and in the second group, the north pole upward (abbreviated as N-magnet group). In the third treatment (control) group, a glass bead of the same weight as the magnet was glued on

the head, and in the fourth treatment (intact) group, toads were only tagged for individual identification. As soon as the treatments were finished, they were released at the site of capture. About 2 minutes were required for each treatment. The magnetic force at the surface of the magnet was 1,800 Gaus. This magnetic force was strong enough to move the pointer of a small magnetic compass put beneath the head portion of a toad bearing the bar magnet.

Tagging of individual toads

Each toad was tagged with a small piece of colored plastic tape. Each piece was serially numbered and glued on the back of each animal. In addition, one of the fore-limbs of each toad in the recapture experiment was banded with an adhesive plaster numbered serially with water resistant ink. The tag on the back lasted at least several days in most cases.

Operations

Toads were blinded by stitching the upper and lower eye lids together with a strong thread. They were thought to be able to distinguish between light and darkness but unable to see objects. For the sham-operation, the needle and thread were just passed through the eyelids. By three to four weeks after the operation, the thread was spontaneously discharged and the toads appeared normal. Toads were also made anosmic by chemically damaging the nasal mucosa. For this purpose, a 5% solution of $AgNO_3$ was poured into the nasal cavity through one of the nostril pits. Successfully operated toads did not respond to vapor of acetic acid. For the sham-operation, a saline solution was poured into the nasal cavity.

Tracking device

To track the migration route of the toads, a colored Nylon sewing thread wound on to a wheel was used (Leona 66, Y-TK2525, Asahi Kasei Sen i Co., Ltd. Tokyo or its equivalent products of other companies), unless otherwise specified. The wheel was put into a loosely fitted plastic cup with a lid and the cup was fixed to the top of a wooden pole of about 30 cm height. The thread came out from a small hole made through the cup wall, and the end of the thread was tied or sewed to a toad. By tracking the thread from the pole, we could find the approximate route of migration of each toad. The tension of the thread was less than 2 g. Usually, we put the thread on migrating toads in the evening and recorded the route the next morning.

Parameters of migration

In the preliminary observation and displacement experiment, the migratory direction of each toad during a given period was defined as follows. A line connecting a point on which a toad was found first (the start point) and the center of the pond was drawn. Another line connecting the start point and the end point of the migration was also drawn. The angle made by these two lines was defined as the orientation or migration angle. The smaller the absolute value of the toad's angle of migration, the better its orientation to the breeding pond.

In the recapture experiment, the recapture rate in the pond in each of the operated, sham-operated and intact groups was calculated and used as the parameter.

In the tracking experiments, a vector composed of the orientation angle and the length of the straight line connecting the start and end points of the migration was used as the parameter of the orientation to the pond. For each experimental group, the mean vector was calculated. At the same time, the standard error was

calculated for both the arithmetic means of the vector angles and lengths of migration of individual animals and used for the statistical analysis. In most experiments, data of toads with their migration distance less than 5 m were omitted.

Statistical analysis

To test whether the distribution of the orientation angle is random or not, the chi-square test for one sample was employed. If the number of individuals was not large enough for the chi-square test, the whole angle range was divided into two intervals, one is the ± 90 degree range around 0 degree (direction toward the pond) and the other is the ± 90 degree range around 180 degree (the opposite direction to the pond) and the distribution was tested by the binomial test under the null-hypothesis of equal distribution. If the random distribution hypothesis was ruled out in a group, i.e. toads migrated in a certain direction, we tested whether or not the arithmetic mean of directions of migration vectors could be deviated significantly from the direction to the pond by the Mann-Whitney U test. In this test, we assumed that directions of the vectors were equally distributed on both sides of the pond direction.

To compare the distribution of the orientation angle between two groups of toads, the chi-square test for two independent samples or the Kolmogorov-Smirnov two sample test was used. Comparison of the recapture rate between two groups was done with Fisher's exact probability test.

RESULTS

Preliminary observation

The first few individuals were found migrating toward the pond on 16th March in the campus of Jiyu Gakuen during the breeding season of 1980. We started the preliminary observation on 17th and continued it until 21st March. Many migrating toads were observed on 17th, 20th and 21st. No toads appeared on 18 and 19th. On 21st, many toads migrated to the pond, but a female toad came out from the pond and started to leave the pond area. Accordingly, we stopped the observation and excluded this individual from data. We recorded the migration routes of 60 toads (17, 22 and 21 on 17th, 20th and 21st, respectively) during this period. The distribution of orientation angles of the 60 toads concentrated around the direction to the pond (Fig. 1). The statistical analysis revealed that the random (uniform) distribution hypothesis had an extremely small probability ($P < 0.01$) of occurring, and consequently it was ruled out.

Changes in the distribution of the migration angle with time were studied by showing the distribution of the migration angle for every 20 min (Fig. 2). The distribution was less clearly concentrated toward the pond at the beginning, from about 18:00 until 19:00. It became sharply concentrated toward the pond direction at the height of migration, between 19:00 and 19:20. The concentration of the distribution became less clear with time after 19:20. The distribution was almost uniform after about 20:00, so the uniform distribution hypothesis could not be excluded after 20:00 ($P < 0.05$). No toads were migrating before 18:00 and after 21:00, except one individual which was active until 21:40 on 20th March. Careful observation of individual
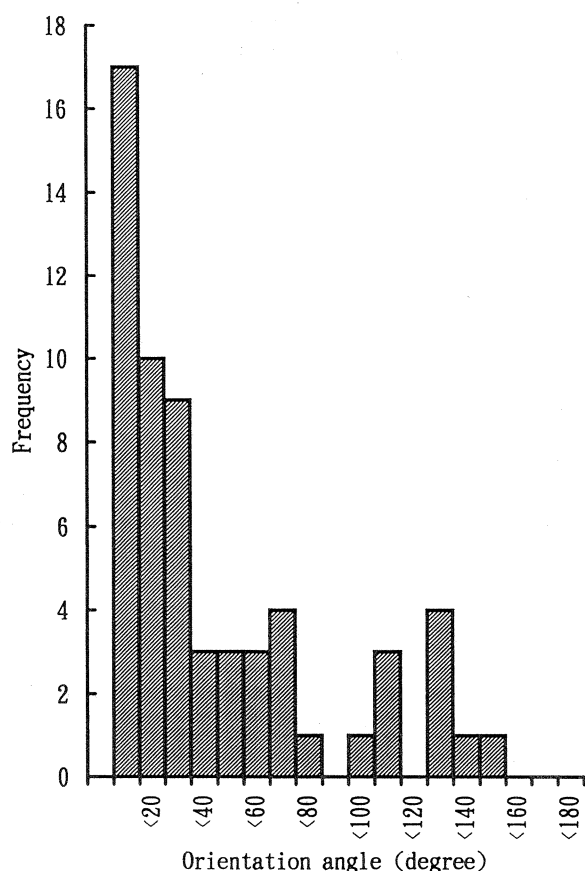
FIG. 1. Distribution of orientation angles (absolute value) of 60 intact toads observed on 17th, 20th and 21st March 1980 at Jiyu Gakuen, Higashi Kurume City, Tokyo. The direction of the breeding pond is 0 degree.

toads revealed that most toads stopped migration toward the pond after about 20:00 and moved around randomly to find a place to hide themselves and to stay until their next active evening.

Displacement experiment

Observation of orientation of intact toads was performed at Heirinji between 12th and 19th in March of 1982, to confirm our findings at Jiyu Gakuen in 1980. Toads migrating toward the pond were observed between 12th and 16th March, and their exit from the pond was observed from 17th. Accordingly, we used data between 12th and 16th and obtained a similar distribution of the migration angle (Fig. 3) to that observed in Jiyu Gakuen (Fig. 1). The uniform distribution hypothesis of the migration angle was ruled out at $P = 2.81 \times 10^{-17}$. A similar change in the migration angle with time was also obtained (data omitted).

In the displacement experiment performed between 21st and 27th in March of 1983, displaced toads to the opposite side of the breeding pond got lost, showing almost random orientations (Fig. 3): the uniform distribution hypothesis could not be ruled out ($P = 0.81$). The control toads which were just carried in a box and returned to their original locations oriented well to the breeding pond (Fig. 3): the

null-hypothesis of the uniform distribution was ruled out at $P = 1.75 \times 10^{-7}$. The difference in the orientation angle distribution between the control and experimental groups was highly significant ($P = 0.00531$).

These data clearly support the third of our working hypotheses: toads use local cues from the migration area or route to orient toward the breeding pond. The first and second hypotheses, using pond cues and celestial cues, respectively, were discarded.

Observation of migrating route from the pond

Twenty-three marked toads were found on the road moving away from the pond. Among these, 6, 9 and 8 were marked red, white and green, respectively, showing that they had passed the respective color zones on the way to the pond a week earlier. Four out of 6 red-marked toads were returning on the red zone and the remaining two on the neighboring white zone (Table 1). All 9 toads marked white were migrating along the white zone. Among 8 green-marked toads, 6 were moving on the green zone and the remaining two on the white and blue zones. No toads marked blue were found on the road during the observation period of three hours. In total, 19 of 23 (82.6%) passed through the same zone when migrating to and from the pond. The remaining 4 toads passed the neighboring zones. We concluded that toads keep the same route on trips to and from the pond.

Recapture experiment with blind and anosmic toads

Experimental toads were found in the pond from 14th March. By 17th March, seven of 20 intact control toads were recovered in the pond, the recapture rate being 35% (Fig. 4). Seven of 20 and 6 of 15 were recaptured in the pond in the blind and its sham-operated groups, respectively. Only 2 out of 24 were recaptured from the anosmic, compared with 7 out of 17 from its sham-operated group. Difference in the recapture rate between the anosmic and its sham-operated group was statistically significant ($P = 0.017$ by the one-sided test). No significant difference in the recapture rate was detected between the blind and its sham-operated groups, between the intact and anosmic sham-operated groups and between the intact and blind sham-operated groups (P values ranging between 0.777 and 0.798). Thus, only anosmic toads showed a significantly low recovery rate in the pond. The two individuals recovered in the pond in the anosmic group were males and no female anosmic was recaptured in the pond. There were no significant differences in the recapture rate between males and females in all 5 groups.

Tracking experiment with blind toads

Migration routes of 5 blind (3 males and 2 females), 7 sham-operated (5 males and 2 females) and 9 intact control toads (5 males and 4 females) were tracked. The route of migration of each toad was expressed as the migration vector (Fig. 5 and Table 2). In all three groups, most toads success-
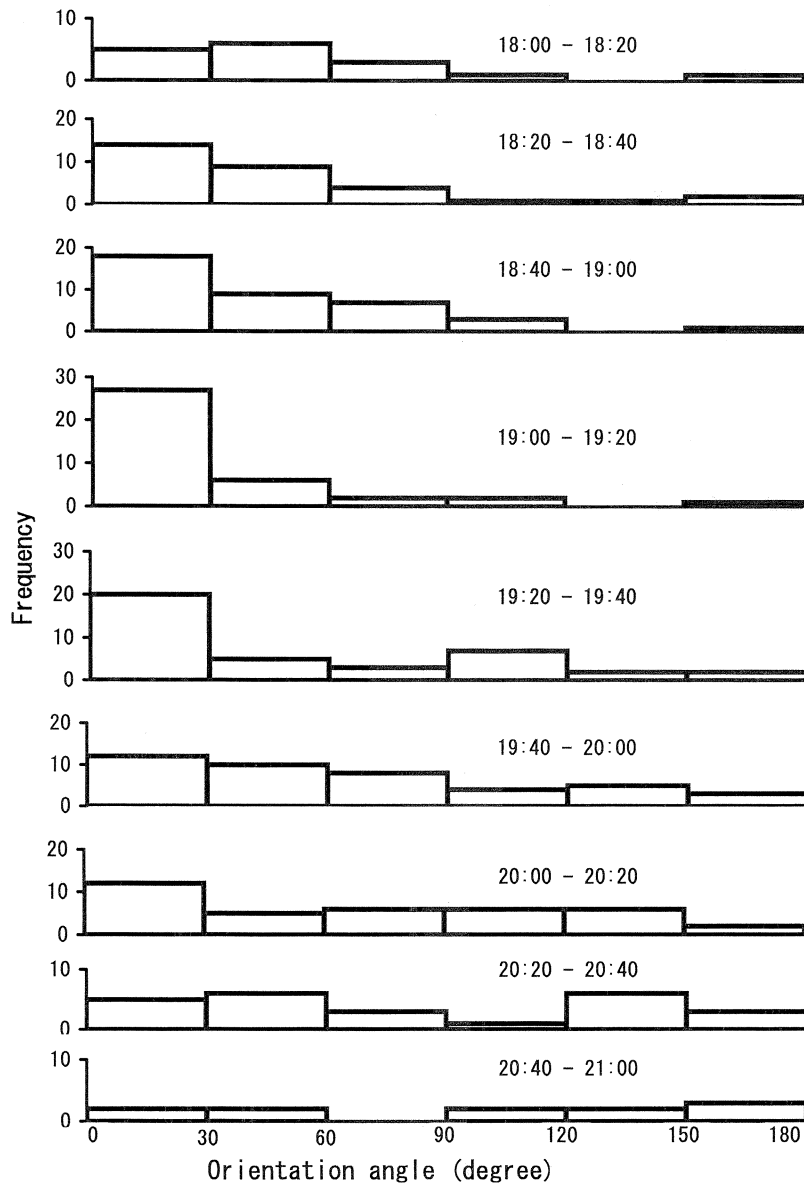
FIG. 2. Change in the distribution of orientation angles (absolute value) with time in 60 toads shown in Fig. 1. The direction of the breeding pond is 0.

fully oriented to the direction of the breeding pond. There were no significant differences in the arithmetic means of individual vector angles and lengths (a straight line connecting the start and end points, representing migration distance) among the three groups ($P > 0.05$). Statistical analyses showed that in the blind and intact groups, the distribution of individual vector angles could not be regarded as random and did not deviate from the direction to the pond. The random distribution hypothesis of the vector direction in the sham-operation group could not be ruled out ($P = 0.25$), due to two individuals with exceptionally large angles. Because of a small number of individuals in each group, males and females were not separately analyzed. In conclusion, all the results indicate that the blindness did not affect the ability to migrate to the pond.

Tracking experiment with anosmic toads

Migration routes of 25 anosmic (9 females and 16 males), 21 sham-operated (7 females and 14 males) and 23 intact (7 females and 16 males) toads were tracked more than 5 m. The migration route of each toad was expressed as the vector as described in the preceding experiment (Fig. 6 and Table 3). The random distribution hypothesis of vector angles could not be ruled out in the anosmic group, while it was ruled out in the sham-operated and intact groups (Fig. 6). In the latter two groups, the arithmetic mean of individual vector directions did not significantly deviate from 0, the direction to the pond. There was no significant difference in the mean lengths of individual vectors among the groups by the analysis of variance ($F = 1.48$, $P > 0.05$), although the mean length of the anosmic group was nominally smaller than
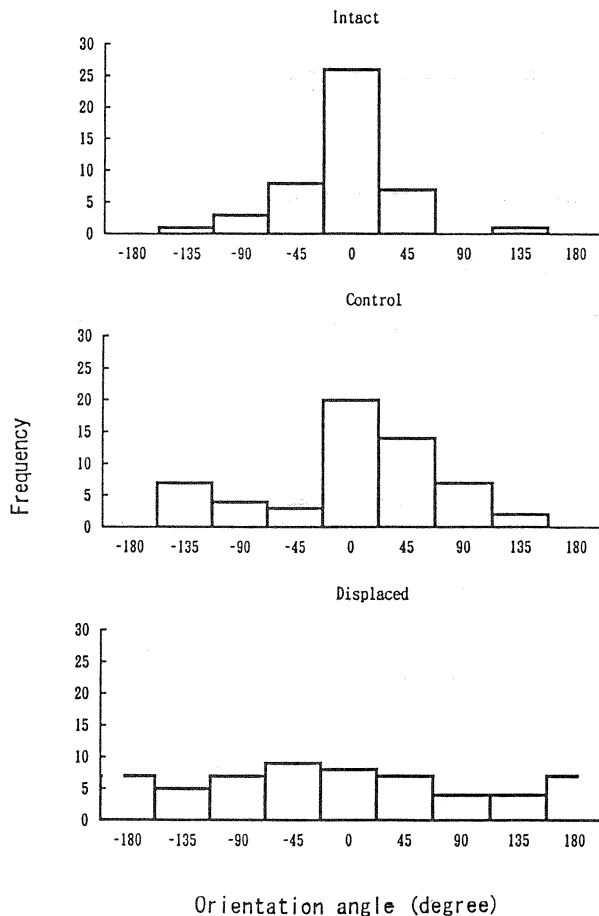
FIG. 3. Distributions of orientation angles of intact toads in March 1981, toads displaced to the opposite side of the pond and control toads moved but returned to the original locations in March 1982.
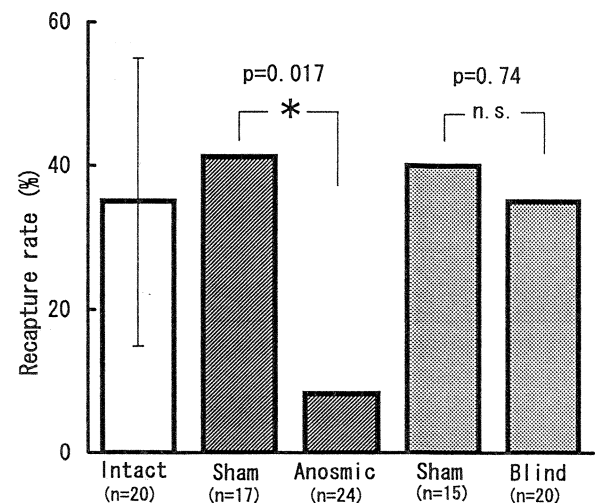

FIG. 4. Recapture rates (%) of intact, anosmic, blind and respective sham-operated toads in the breeding pond. The 98% confidence interval for the recapture rate in the intact group is indicated with a bar. The recapture rates in the anosmic and blind groups were compared with those of corresponding sham-operated groups by Fisher's exact probability test and p values of the test are indicated in the figure.

the others.

We thus concluded that anosmic toads were completely disoriented, whereas the sham-operated and intact control toads oriented well to the breeding pond. The locomotion activity of the anosmic toads was not significantly affected.

Tracking experiment with toads bearing a magnet

The migration of 21, 17, 22 and 17 toads were tracked in the S-magnet, N-magnet, control and intact groups, respectively. The number of toads which did not move or were lost was 3, 6, 1 and 6 in the respective groups. The route of migration of each toad was expressed as the migration vector (Fig. 7 and Table 4). In all the four groups, most toads successfully oriented to the direction of the breeding pond.

There were no significant differences in the arithmetic means of individual vector angles and lengths among the four groups ($P>0.05$). Statistical analyses showed that the distribution of individual vector angles could not be regarded as random and did not statistically deviate from the direction of the pond in any of the groups. Since there was no significant difference in either the vector angle or length of migration between males and females, they were not differentiated in the above analyses. We concluded that the bar magnet has no effect on the proper orientation to the pond.

DISCUSSION

Our preliminary experiment showed that the locomotor activity of *Bufo japonicus* on the way to the breeding site consisted of two parts; one is the active migration toward the pond and the other is random movement to search for a hiding place, which occurs near the end of the night activity. To study the orientation toward the pond, the latter movement should be excluded, although it is practically difficult. The effects of the random movement on the analysis of the migration were minimized by limiting the observation hour and excluding data representing movement over too short a distance.

TABLE 1. Distribution of returning toads crossing 4 different zones on which they had crossed on the way going to the pond

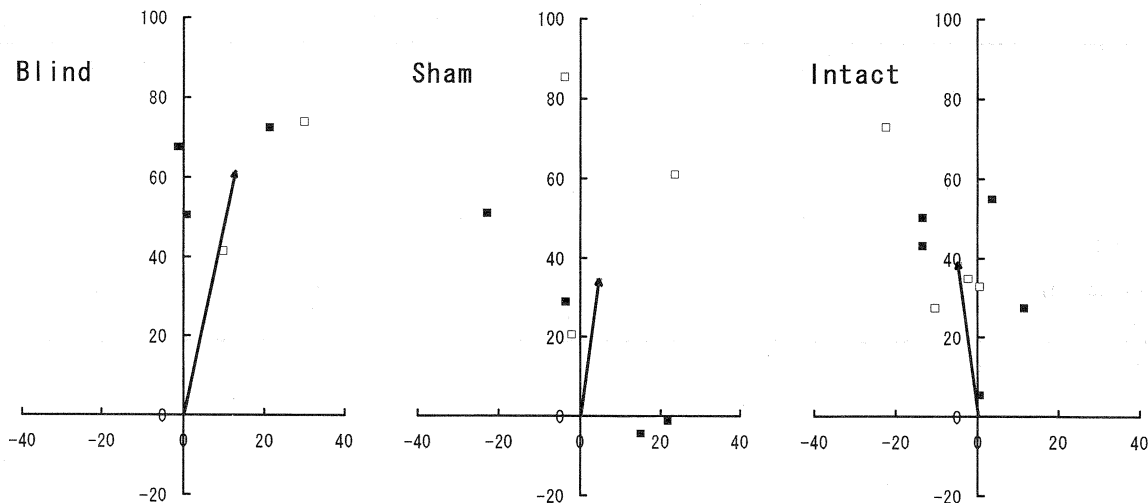| Zones crossed Number	Red 6				White 9				Green 8			
Zones crossing	Red	White	Green	Blue	Red	White	Green	Blue	Red	White	Green	Blue
Number	4/6	2/6	0/6	0/6	0/9	9/9	0/9	0/9	0/8	1/8	6/8	1/8

FIG. 5. Terminal points of vectors (small squares) representing migration of blind, sham-operated and intact toads captured at locations 50 to 100 m from the breeding pond, immediately operated and released soon after the operations. The initial point of each vector is taken at the origin of the axes, and the direction of the pond is 0 degree (the upward of the Y-axis). Empty and filled squares represent females and males, respectively. The mean vector is also indicated with an arrow. The unit of the axis values is m.

TABLE 2. Mean vector and distance of migration in toads of the blind, sham-operated and intact groups

Group Number of indiv.	Blind 5 (2)*	Sham-operated 7 (3)	Intact 9 (4)
Mean vector			
Angle (°)	11.3	6.8	−7.4
Length (m)	62.4	34.7	39.0
Mean distance (m)	63.2	42.0	40.0
SEM of mean dist.	7.1	27.9	20.5

* Number of females is given in parentheses.

Displacement experiment has been a favorite method of studying orientation of amphibians [2, 16, 22]. The objective of their passive displacement experiments was to know whether normal or operated toads can orient to a certain direction or not. However, the present displacement experiment is considerably different from those used by the previous investigators. In the present study, we designed our displacement experiment to test the three hypotheses at once, i.e. using cues originated from 1) the pond, 2) celestial bodies or 3) the local area or route of migration. Then, we transported migrating toads to the opposite side of the pond. Since the displaced toads got lost and could not orient to a certain direction, we concluded that they used a cue originating from the local area or route of migration to orient to the pond. This experiment excluded the possibilities using the pond and celestial cues.

Our tracking experiments and recapture experiment clearly showed that toads used the olfactory sense to follow the route to the pond. Similar results showing disorientation
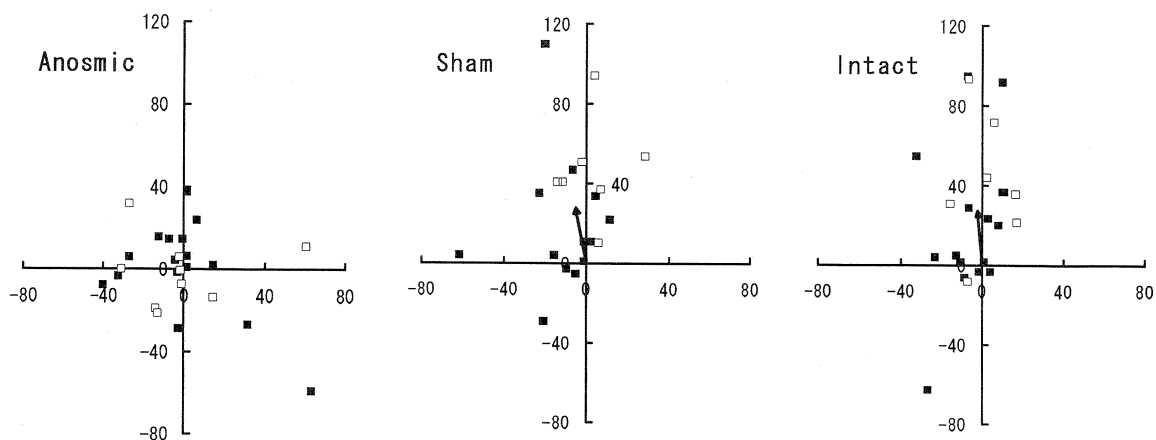

FIG. 6. Terminal points (small squares) of vectors representing migration of anosmic, sham-operated and intact toads. They were captured at locations 50–200 m from the breeding pond, made anosmic or sham-operated in the laboratory, kept at 5°C and released at the original locations on the next relatively warm and humid evening. The initial point of each vector is taken at the origin of the axes, and the direction of the pond is 0 degree (the upward of the Y-axis). Empty and filled squares represent females and males, respectively. The mean vector is indicated with an arrow. The unit of the axis values is m.

TABLE 3.   Mean vector and distance of migration in male and female toads of the anosmic, sham-operated and intact control groups

| Group | Anosmic | | | Sham-operated | | | Intact | | |
Sex	Male	Female	Comb.	Male	Female	Comb.	Male	Female	Comb.
Number	16	9	25	14	7	21	16	7	23
Mean vector									
Angle (°)	98.8	−135.5	−159.5	−31.2	1.75	−14.4	−14.2	2.60	−6.41
Length (m)	0.070	1.400	0.549	17.15	41.51	25.28	20.18	41.1	26.55
Mean distance (m)	26.88	23.77	25.77	29.58	48.68	36.58	33.14	45.87	37.01
SEM	5.04	6.47	3.91	8.01	9.50	6.54	5.47	6.28	5.41

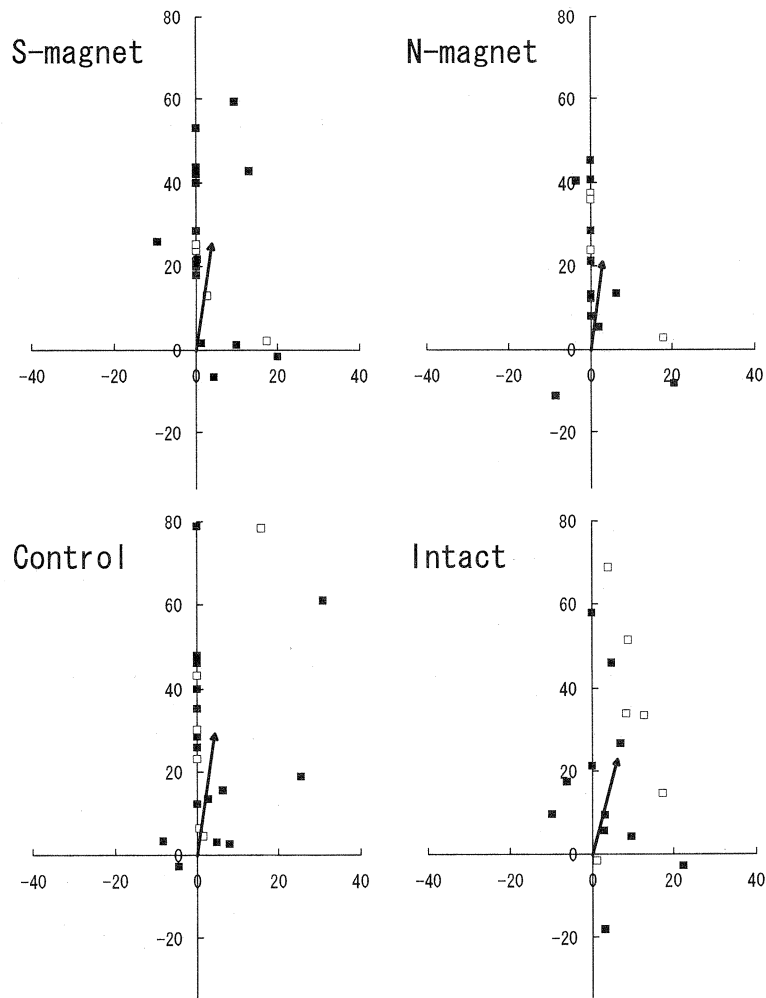Comb.=males and females combined

FIG. 7.   Terminal points (small squares) of vectors representing migration of toads bearing a bar magnet on the head, control toads bearing a glass bead and intact toads.   The toads were captured at locations 50 to 100 m from the breeding pond, a magnet, a glass bead or nothing glued on the head and released immediately at locations of capture.   The initial point of each vector is taken at the origin of the axes.   The direction of the pond is 0 degree (the up-ward of the Y-axis).   The mean vector is also indicated with an arrow.   Empty and filled squares represent females and males, respectively.   The unit of the axis values is m.

of anosmic toads have been reported [16, 22].   The results of the present displacement experiment and tracking experiments lead us to the conclusion that *B. japonicus* uses the olfactory cue originating from the route or area of migration and not that from the pond.   Sinsch [18] described that a

similar strategy for orientation was used in salamanders, i.e. homing salamanders used local odor patterns.

We further found that toads moved approximately the same route or area of migration both to and from the pond. If we extrapolate this fact to the past, toads should have

TABLE 4. Mean vector and distance of migration in toads with a bar magnet (S-magnet and N-magnet groups), a glass bead (control group) and tagged only (intact group)

Group Number of indiv.	S-magnet 21 (4)*	N-magnet 17 (4)	Control 22 (6)	Intact 17 (6)
Mean vector				
Angle (°)	7.61	5.69	7.78	13.55
Length (m)	24.84	20.35	28.20	22.79
Mean distance (m)	27.73	24.53	30.03	25.57
SEM	0.72	0.72	1.03	1.09

* Number of females is given in parentheses.

moved through the same route or area when they first migrated from the pond soon after their metamorphosis. Accordingly, it is probable that postmetamorphic toadlets memorize the route or area of their migration with the olfactory sense and it is imprinted into their developing brain. After two to three years, on a warm and humid night in the early spring they suddenly become aware of the route or area and start their breeding migration, using the olfactory memory. This proposed memorizing mechanism in the toad resembles that in the wellknown studies on the migration of salmon (see for review [8]). In the salmon, the imprinting of the odor of the home river occurs at the smolt stage when thyroid hormone is actively secreted and the down stream migration is occurring. It is well known that a thyroid hormone surge takes place in metamorphosing amphibians and hence, in the toad, the imprinting of the olfactory map is considered to take place when thyroid hormone secretion is still high. However, there is a difference between these two species, i.e. the salmon memorizes an odor of the home river, but the toad presumably memorizes odors along the route or in the area of migration and not that of the home pond.

In the salmon, it was reported that sex steroid hormones are actively secreted at the time of the upstream migration for breeding and the hormones modify olfactory sensitivity to increase discrimination capacity. In the Japanese toad, it has been reported that plasma levels of sex steroid hormones are high through the breeding migration period [9]. Accordingly, it is probable that sex steroid hormones increase olfactory sensitivity in the Japanese toad, as they do in the salmon. Nakazawa et al. [13] recently reported that a marked oscillatory electric activity that was superimposed on the typical electro-olfactogram pattern was induced by an artificial stimulation in the olfactory mucosa in both sexes of the Japanese toad. This oscillatory activity could be induced only in the breeding season. It became detectable around the time when the breeding migration started and lasted until the time when the post breeding migration from the pond ended. The physiological correlation between this electric activity and orientation should be clarified.

Our release and recapture experiments using blind toads indicate that blind toads can properly orient to the breeding pond. It has already been reported in several species of

Bufo that blindness did not affect orientation to the home pond [2, 7, 16, 22]. Accordingly, in *Bufo japonicus* also, optical visual sense is not necessary for pond orientation, but a possibility that the pineal complex plays a role in the orientation remains to be examined.

The olfactory orientation in *Bufo japonicus* is different from that in the other toads reported by previous investigators [16, 22]. The previous investigators suggested odor from the pond as the cue in several species of *Bufo* except *B. spinulosus* [17], but the cue in *B. japonicus* is considered not originated from the pond. It was reported that *B. bufo* gathered at the site of an extinct pond (see Sinsch [18]). We have also made a similar observation (Ishii, unpublished): the pond in the Jiyu Gakuen campus was completely removed by excavating the ground for construction of a new building in January of 1981, but a number of toads gathered in March of 1981 at the place where the pond had been and a less number returned in the spring of 1982. These observations strongly support the conclusion that toads are not attracted by the cue from the breeding pond.

Sinsch [16] reported that a bar magnet put on the head affected the orientation of *B. bufo* to the breeding site. However, our displacement experiment ruled out the possibility of the celestial cue. On the other hands, the results of our tracking experiment showing that anosmic toads lost the orientation to the breeding site are in good agreement with a similar experiment by Sinsch [16]. If we theorize that toads use the magnetic cue at the initial stage of the migration to the pond and then the olfactory cue in the later stage of the migration, the results of those of the present study and those of Sinsch become concordant, because there is a difference in the distance of the release point from the pond between these two studies, 145 to 200 m in Sinsch s and 50 to 100 m in ours. Another possible explanation is a map-based orientation using two different cues suggested by Sinsch [18]. If toads combine both the magnetic and olfactory cues, such as memorizing a certain compass direction in combination with a certain sort of odor and changing the direction according to the nature of the odor, either the anosmic or blind operations could affect the orientation. This might be an attractive theory.

However, our results from the magnet experiment ruled out the possibility of the magnetic orientation: a bar magnet glued on the head of *B. japonicus* did not affect the orientation to the breeding pond at all. The strategy employed by *B. japonicus* may be a map-based orientation using either a combination of the local olfactory and some unknown cues or simply a local odor pattern as suggested in salamanders. We prefer the latter hypothesis, i.e. the local odor-map hypothesis, because it is simpler and none of the present results conflicts to it.

Thus, we proposed a simpler mechanism of the orientation to the breeding pond in *B. japonicus* than the other investigators did in the other species of *Bufo*. The reason of the difference may be that we employed a simple approach: making a clear hypothesis or hypotheses on the mechanism of

the orientation first and then designing experiment(s) to examine the hypothesis or hypotheses. We also repeated this procedure by changing the hypothesis from less specific to more specific one. Furthermore, we employed only field experiments but laboratory experiments, because there is a possibility to observe false migratory behavior in the laboratory experiments. Interestingly, most results supporting the sun compass theory were derived from experiments with toads transported to the laboratory and kept in an artificial pen, arena or pool [6, 12, 21]. In contrast, all the field experiments with toads unanimously supported the olfactory and/or visual orientation [5, 16, 19, 22]. We should be careful to evaluate movements of toads under the laboratory conditions in studies on the orientation to the breeding site.

Sinsch [17] reported in one of his reviews that 6 of 7 species of *Bufo* used the olfactory cue for the orientation. However, some results of these field studies are difficult to explain simply from the olfactory cue as pointed out by Sinsch [17]. This may be due to that all these investigators supposed the pond-originated cue. If they pay attention to the local cue from the migration route, different experimental designs should had been used and different results could be obtained.

ACKNOWLEDGMENTS

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