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RESEARCH ARTICLE

Internal state transition to switch behavioral strategies in cricket phonotaxis

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ABSTRACT
Animals employ multiple behavioral strategies for exploring food and mating partners based on both their internal state and external environment. Here, we examined how cricket phonotaxis, which was considered an innate reactive behavior of females to approach the calling song of conspecific males, depended on these internal and external conditions. Our observation revealed that the phonotaxis process consisted of two distinctive phases: wandering and approaching. In the latter phase, crickets moved directly towards the sound source. The transition into this phase, referred to as the 'approach phase', was based on changes in the animal's internal state. Moreover, retention of the approach phase required recognition of the calling song, while song loss downregulated cricket mobility and induced frequent stopping. This is a typical movement in local search behaviors. Our results indicate that phonotaxis is not only a reactive response but a complicated process including multiple behavioral strategies.

KEY WORDS: *Gryllus bimaculatus*, Approach phase, Insect, Local search, Mating behavior, Auditory

INTRODUCTION
Animals must employ multiple behavioral strategies to adapt to different situations, such as foraging, mating and homing (Hills et al., 2015). These behaviors are selected based on the internal state of animals, as well as their external environment (Nevitt, 2008; Sternson, 2013). For example, the internal state, including nutrition and mating status, modulates the area in which the animals forage by altering exploration and exploitation decisions (Corrales-Carvajal et al., 2016). For the external environment, animals restrict their search area once they detect olfactory cues associated with food (Nevitt, 2000). In some cases, decreased quantity and quality of surrounding food modulates their behavior to change the frequency of turns and forward movement (Calhoun et al., 2015; Gray et al., 2005). These behavioral strategies are optimized for the situation by changing the movement of animals and contributing to their survival.

Field crickets exhibit prominent goal-directed behavior responding to a sound cue for mating, phonotaxis, in which a female cricket approaches conspecific males that are singing a calling song. Cricket phonotaxis has been studied as a classical model in neuroethological research (Huber et al., 1989). Various behavioral experiments have been conducted in an arena or by using a spherical treadmill system to reveal the essential components of phonotaxis (Bailey and Thomson, 1977; Hedwig, 2006; Rheinlaender and Blätgen, 1982; Schöneich and Hedwig, 2010; Thorson et al., 1982; Weber et al., 1981). The tympanal organs positioned on the forelimb of crickets receive the calling song, and the acoustic information is transferred to the brain via ascending auditory neurons identified within the thoracic ganglia (Hedwig and Stumpner, 2016). Females move towards the males by detecting the acoustic features of the calling song, including sound frequency and patterns (Thorson et al., 1982). The interneurons of the brain extract the song-specific acoustic characteristics, which enables the crickets to recognize the conspecific calling song (Kostarakos and Hedwig, 2012). The ability to distinguish small changes in the orientation of the sound suggests highly accurate directional processing in the cricket nervous system (Schöneich and Hedwig, 2010).

These previous studies described the instantaneous response to the calling song at the behavioral level. Studies using the more sophisticated treadmill system indicated that crickets were able to respond to the calling song within milliseconds (Hedwig and Poulet, 2004; Poulet and Hedwig, 2005), suggesting that phonotaxis is only a reactive behavior to the calling song. In contrast, some research in which cricket phonotaxis was observed in an experimental arena demonstrated different phases in the phonotaxis process through their trajectory (Bailey and Thomson, 1977; Mhatre and Balakrishnan, 2007; Rheinlaender and Blätgen, 1982). These results indicate that crickets may have multiple distinctive phonotaxis strategies rather than a simple approach behavior that is just reactive to the calling song.

To examine whether crickets employed multiple behavioral strategies based on their internal state and external environment, we observed crickets moving freely within a circular arena where the conspecific calling song was emitted from loudspeakers on the wall. The trajectory of the crickets revealed that the phonotaxis process consisted of distinctive phases based on internal state. Meanwhile, experiments on song timing and sound-source location indicated that the crickets could adapt their behavior to external conditions when approaching a sound source. These results suggest that phonotaxis is a complicated behavioral process in which multiple strategies are adopted.

MATERIALS AND METHODS
Animals
Laboratory-bred adult female crickets (*Gryllus bimaculatus* De Geer 1773) 2–3 weeks after imaginal molting were used throughout our experiments. The body mass of the crickets ranged from 0.7 to 1.4 g. They were reared under 12 h:12 h light:dark conditions at a constant temperature of 28°C and could freely access food and water during the larval stages. To isolate them from male crickets, all

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experimental animals were transferred from a rearing room to an incubator during the eighth instar larval stage. The rearing conditions in the incubator were the same as those in the rearing room except the temperature was 27°C. The guidelines of the Institutional Animal Care and Use Committee of the National University Corporation, Hokkaido University, Japan specify no requirements for the treatment of insects in experiments.

Experimental apparatus

All experiments were conducted during the dark phase at room temperature (21.5–26.5°C) on a specially constructed circular arena (1 m diameter; Fig. 1A) in a sound-attenuated room (AMC-3525, O'Hara & Co., Ltd, Tokyo, Japan) where anechoic foam sheets (F2-PF, Strider, Toyohashi, Japan) were attached to the ceiling and inner walls. All experiments were conducted under red LED illumination where it was darkness for crickets. A cricket was monitored from above the arena at 30 frames per second using a USB3.0 CMOS camera (DN3RG-130BU, Shodensha, Osaka, Japan) mounted on the ceiling of the sound-attenuated room (Fig. 1A). Crickets were marked with two white spots (3 mm diameter) on the dorsal surface of the thorax and wing to automatically detect cricket location and body axis on video images. A cricket was placed within a plastic cylinder, which was installed at the center of the arena and remote-controlled by air pressure from outside the sound-attenuated room. After the cylinder dropped under the floor, which allowed the cricket to move, an acoustic stimulus was immediately emitted. The

A

Camera Ceiling speaker

1 m

Wall speaker

Anechoic foam

Start position

Air-pressure-controlled cylinder

B

Chirp

Fig. 1. Experimental systems. (A) Experimental apparatus for monitoring cricket phonotaxis. A cricket started to move at the center of the arena. A loudspeaker was installed on the arena wall and another on the ceiling of the soundproof room. Cricket movement was monitored with a digital video camera installed on the ceiling. (B) Oscillogram of the chirps used as acoustic stimulation; scale bar, 100 ms.

movement of the cricket was then recorded until it arrived at the arena wall. The resolution of the obtained image was 0.957 mm pixel^{-1}, and the image size was 1280×1024 pixels, which covered the whole arena. With a custom-made program written in C++ with OpenCV, the markers on the animal were automatically traced from each image, and the positions were recorded online. All recordings and manipulations were conducted from the sound-attenuated room.

Acoustic stimulation

The acoustic stimulus of the calling song was synthesized using a single chirp recorded from a male of the same species in the same colony in the laboratory (Fig. 1B). The chirps including four syllables were repeated every 320 ms. The duration of each syllable was approximately 31 ms. The artificially edited calling song and white noise were made using a python program and presented by 1.5-inch (38.1 mm) full-range scale loudspeakers (MM-SPS2, Sanwa Supply, Okayama, Japan). The sound pressure level and the speaker emitting the sound were controlled via a volume controller and electrical switcher controlled by Arduino Due (Arduino LLC). A speaker was installed on the arena wall (at 150 mm height) so that the center of the speaker was positioned 35 mm from the arena floor. An additional composite speaker (MM-SPL6, Sanwa Supply), which consisted of a 2.5-inch (63.5 mm) full-range scale loudspeaker and 1.0-inch (25.4 mm) tweeter, was installed on the ceiling of the sound-attenuated room so that the center of the speaker was aligned with the center of the arena. The distance from the ceiling speaker to the arena floor was 145 cm. The sound pressure level (SPL) of the wall speaker was measured at a distance of 20 cm with a microphone (B6, Countryman Associates, Menlo Park, CA, USA) and sound interface (Octa-Capture UA-1010, Roland, Hamamatsu, Japan), which was also calibrated with the BK microphone (5935, Brüel & Kjær, Nærum, Denmark) in advance. The exact SPLs for the acoustic stimulation labeled as 60, 70 and 80 dB were 59.3, 71.6 and 82.7 dB, respectively. *In situ* SPL at the approach points was calculated based on the distance from the wall speaker and the SPL of the acoustic stimulus as follows:

$$\mathrm{SPL}_{\mathrm{cricket}} = \mathrm{SPL}_{20} - 20 \times \log\left(\frac{r_{\mathrm{cricket}}}{20}\right), \quad (1)$$

where $\mathrm{SPL}_{\mathrm{cricket}}$ is the sound pressure level at the approach point and r_{cricket} is the distance from the wall speaker to the cricket at the approach point. SPL_{20} is the sound pressure level measured 20 cm away from the wall speaker. The *in situ* SPLs calculated from the above formula corresponded to the actual measurement values. The SPL of the ceiling speaker was also calibrated on the arena floor at the center of the arena using the microphone and sound interface. To avoid sound reverberation, the anechoic foam was also attached to the inside of the arena wall except the speakers.

Experimental procedure

We performed four types of experiments with different stimulation methods. For each trial in all types of experiments, the video recording was started when a plastic cylinder was dropped and the cricket could move from the center of the arena, and finished when the cricket reached the arena wall at any location. In the first experiment (Figs 2 and 3; Fig. S1), white noise (WN) and the calling song (CS) at different sound intensities were consistently emitted by the speaker on the wall from the beginning of movement at the center of the arena until the cricket arrived at the wall. At first, to adapt the cricket to the experimental conditions, the cricket freely
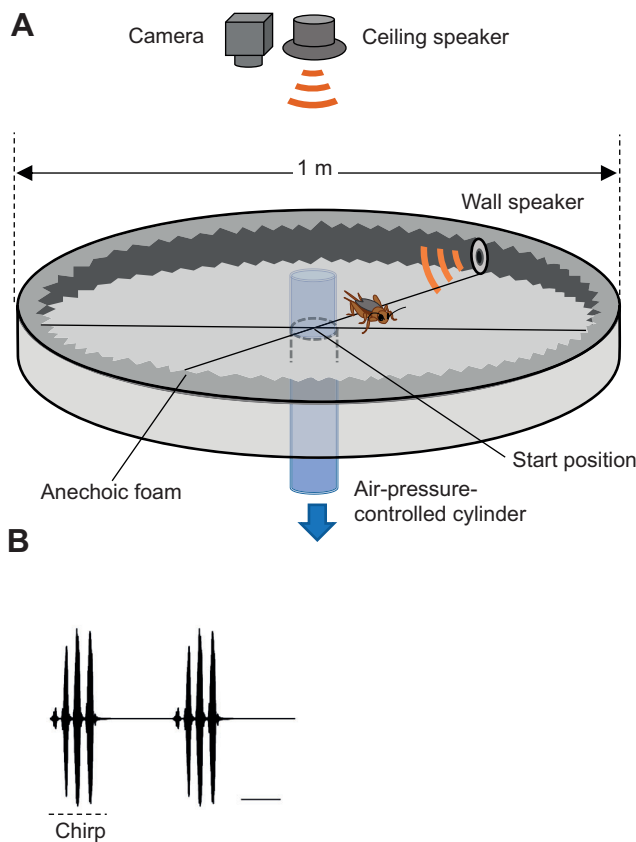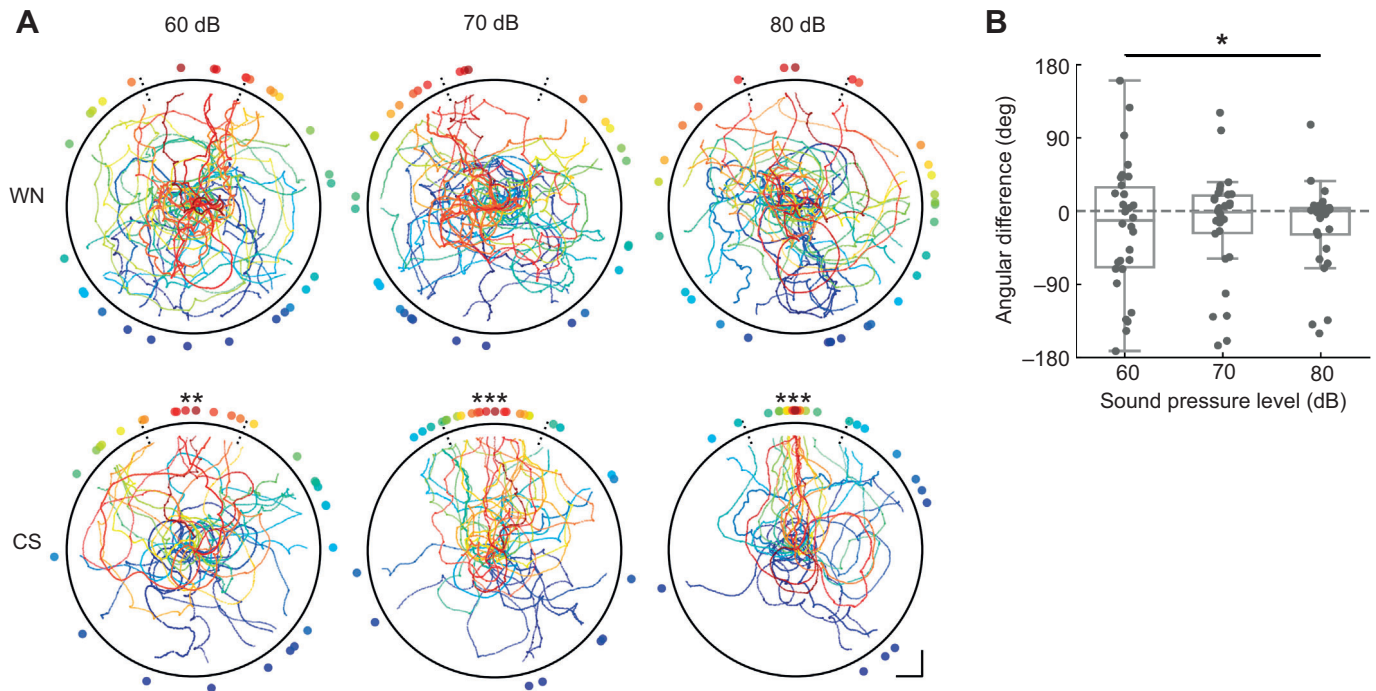
Fig. 2. Phonotaxis of the crickets freely moving in the arena. (A) Trajectories of individual crickets at different sound intensities. White noise (WN) or calling song (CS) was presented at the sound pressure levels indicated at the top of the panels. The speaker was located at the top of each image. Colors represent different individuals. Filled circles outside the arena showed the locations where crickets arrived at the wall. Dotted lines indicate ±22.5 deg from the line connecting the speaker and the center of the arena. Scale bar, 10 cm. $N=30$ individuals for each SPL. (B) Angular differences between the arrival points and the speaker presenting the calling song. Gray dots indicate the values for each individual. A V-test (A) and a Wallraff test with Bonferroni correction (B) were used; significant differences: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

moved in the arena where the 80 dB white noise was emitted until it reached the arena wall. The 60, 70 or 80 dB calling songs were then emitted, and finally white noise at the same SPL as the calling song was emitted again to obtain the control data. In total, three trials were performed for each individual cricket. Thirty individuals for each sound pressure level were tested in the first experiment.

In the second experiment (Figs S1 and S5), the sound stimuli were presented at 70 dB from the ceiling speaker in advance, and the following sounds were presented from the wall speaker at 60 dB. Under the WN in advance (WN_{ad}) and CS in advance (CS_{ad}) conditions, the white noise or calling song was presented for 1 min during which a cricket was confined within the cylinder at the center of the arena. Under CS for control (CS_{ctl}) and WN for control (WN_{ctl}) conditions, the cricket was also confined in the cylinder for 1 min during which no sound was emitted. Under all conditions, as soon as the cricket was released into the arena, the white noise or calling song was emitted from the wall speaker. The trial under the WN_{ctl} condition was conducted first, and the trials under the other conditions were conducted in a random order. Thus, four trials for WN_{ctl}, CS_{ctl}, WN_{ad} and CS_{ad} conditions were performed for each individual cricket. Thirty individuals for each condition were tested in the second experiment.

In the third experiment (Fig. 4; Fig. S1), the sound stimulus or sound-source location was switched when the cricket reached 30 cm away from the center of the arena. This criterion of the switching position was decided from the result of the first experiment. The sound pressure level emitted from the wall speaker was 80 dB, and that from the ceiling speaker was 70 dB. In the first trial, to adapt the cricket to the experimental conditions, the white noise was consistently emitted from the wall speaker throughout their

trajectory, which was also the negative control referred to as the WN_w (WN from wall)–WN_w condition. Then, the trials under the subsequent three conditions were conducted in a random order (Fig. S1). Under the CS_w (CS from wall)–WN_w condition, the calling song was switched to white noise, both of which were emitted from the wall speaker. Under the CS_w–CS_c (CS from ceiling) condition, the sound-source location was switched from the wall speakers to the ceiling speaker, both of which emitted the calling song. Under the CS_w–CS_w condition, the calling song was consistently emitted from the wall speaker throughout their trajectory, which was a positive control. Thus, four trials for WN_w–WN_w, CS_w–WN_w, CS_w–CS_c and CS_w–CS_w conditions were performed for each individual cricket. Thirty individuals for each condition were tested in the third experiment.

In the two types of the fourth experiment (Fig. 5; Fig. S1), the sound stimulation was changed in the time or sound-source location depending on cricket movement, which was running or stopping (standing still). In short, crickets' running or stopping resulted in the change of the sound stimulus. Classification of the movements was based on the translational velocity. If the cricket moved faster than 15 mm s^{-1}, then this movement was defined as running. If the moving speed was slower than 15 mm s^{-1}, then that was defined as stopping. In the intermittent experiment, the 80 dB calling song was emitted from the wall speaker only during running or stopping, which were referred as the CS_{run} (CS during running) and CS_{stp} (CS during stop) conditions, respectively (Fig. 5A). In the complementary experiment, the calling song was emitted throughout the trial, but the sound-emitting speaker was immediately switched depending on cricket movement. The SPLs of calling song from the wall speaker and the ceiling speaker were
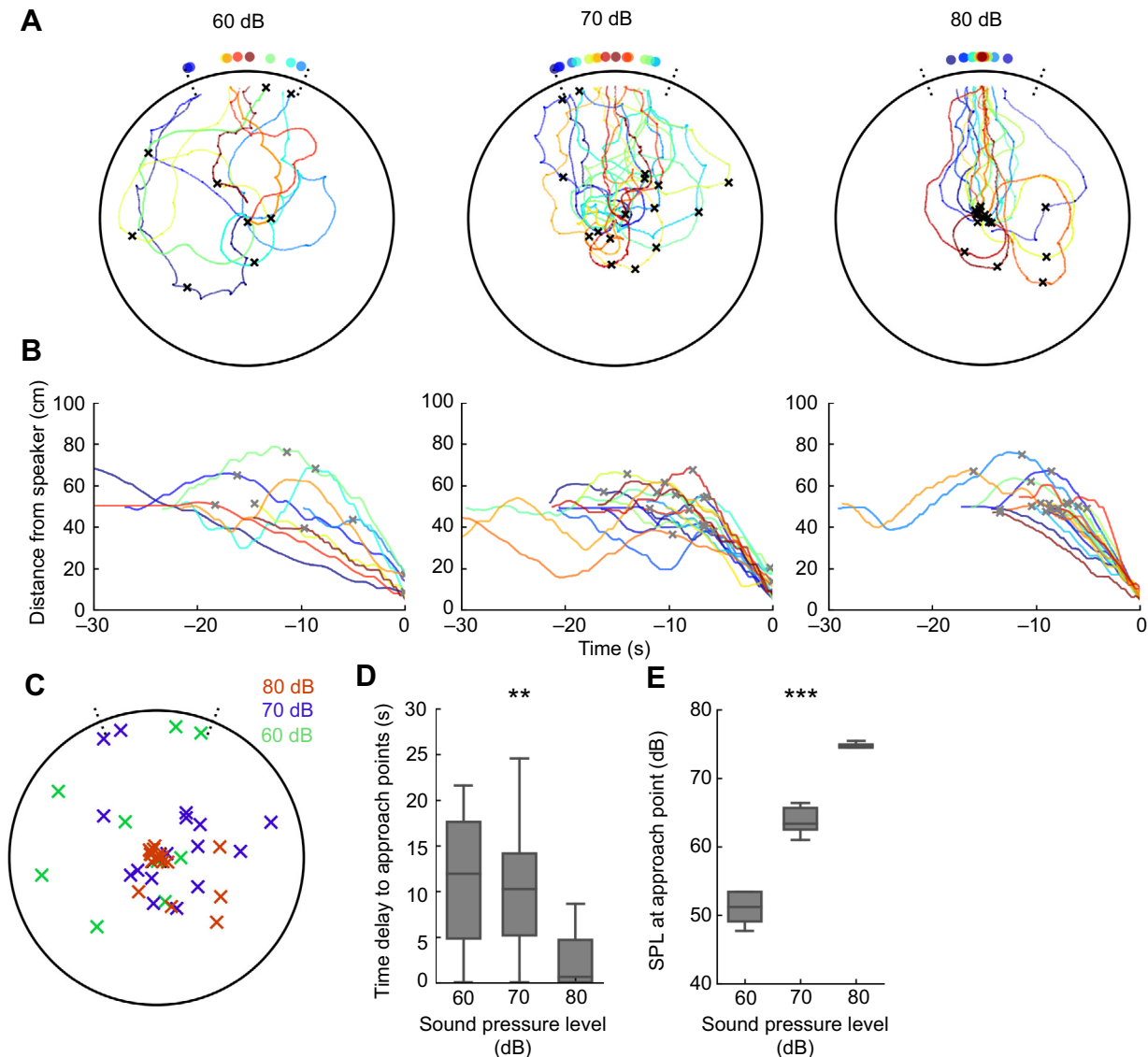
Fig. 3. Two distinct behavioral phases in phonotaxis of localized crickets. (A) Trajectories of crickets localized within 22.5 deg from the speaker in Fig. 2; for details, see legend of Fig. 2. Black crosses indicate the approach points where the approach phases started. N=9, 16 and 17 individuals for 60, 70 and 80 dB, respectively. (B) Time course changes in the distance between crickets and the speaker. Time 0 denotes the finish point of each trial. Gray crosses indicate the approach points when the approach phases started. (C) Locations of the approach points in the arena. Colors represent SPL. (D) Time delays from the start to the approach points for different stimulus intensities. (E) *In situ* sound pressure levels at the approach points for different stimulus intensities. One-way ANOVA (D) and Kruskal–Wallis rank tests (E) were used; significant differences: **$P < 0.01$, ***$P < 0.001$.

80 dB and 70 dB, respectively. Under the CS_{run} condition, the song was emitted either by the wall speaker while running or by the ceiling speaker while stopping. Under the CS_{stp} condition, the relationship between the sound source and cricket movement was reversed (Fig. 5A). In both experiments, to adapt the cricket to the experimental conditions, white noise was consistently emitted from the wall speaker throughout the first trial, which was also the negative control referred to as the WN condition. Then, the trials under the CS_{run} and CS_{stp} conditions were conducted in a random order. In the final trial, the calling song was consistently emitted from the wall speaker, which was a positive control (CS for control, CS_{ctl}). Seventy-eight and 74 individuals from different groups were tested in the intermittent and complementary experiments, respectively. The sound pressure level from the wall speaker was 80 dB and that from the ceiling speaker was 70 dB.

Behavioral analysis

We analysed cricket movement based on the x- and y-coordinates of the two markers automatically detected on each video image. The trajectory was determined from the coordinates of a thoracic marker. When the translational velocity exceeded 15 mm s^{-1}, the cricket was considered to be running; otherwise it was stopping (standing still). The duration of running was defined as a running bout. The accuracy of the sound localization for each trial was evaluated as the angular difference between the wall speaker emitting the sound stimulus and the finishing points where the crickets arrived at the wall. The mean velocity was calculated as the average value of the translational speed measured only in the running bout. The ratio of running was determined as the proportion of running to the total time from start to finish in each trial. The distance per bout was the travel distance for each running bout. The number of times that
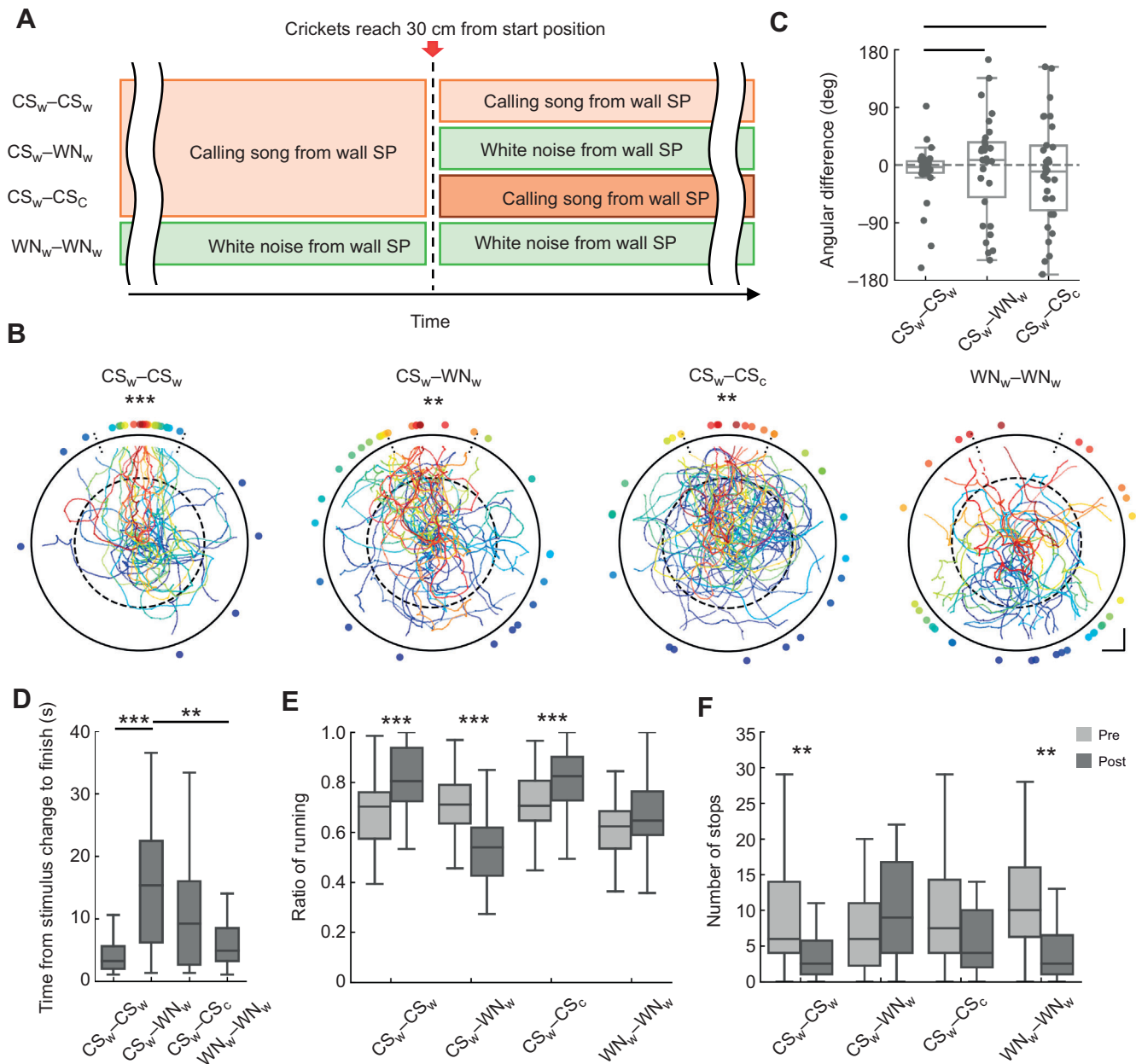
Fig. 4. Effects of the loss of the calling song on phonotaxis. (A) Temporal scheme of the song-loss experiments. The contents of acoustic stimulation (CS or WN) or the sound-source location were switched when the crickets reached 30 cm from the center of the arena. SP, speaker. (B) Trajectories of individual crickets. Dashed circles indicate 30 cm away from the center of the arena. The other descriptions are the same as in Fig. 2A. N=30 individuals. CS_w, calling song from wall; CS_c, calling song from ceiling; WN_w, white noise from wall. (C) Angular differences between the arrival points and the speaker. Gray dots indicate the values for each individual. (D) Time from stimulation switching to finish. (E,F) Temporal ratio of running period (E) and number of stops (F) before (Pre) and after (Post) the stimulation switching. A V-test (B), Wallraff test with Bonferroni correction (C), Wilcoxon rank sum test with Bonferroni correction (D) and Wilcoxon signed rank test (E,F) were used; significant differences: **$P<0.01$, ***$P<0.001$.

stopping occurred referred to the total number of interruptions between running bouts in each trial.

The approach points were determined by the time variation in the approach velocity (Fig. S2A), which was calculated as the time derivative of the distance between the speaker and the thoracic marker on the cricket at each frame. At first, we examined the distribution of the approach velocity for the crickets that were exposed to the calling song of 60, 70 and 80 dB (Fig. S2B). We observed two distinct peaks in the distribution for the datasets of all tested crickets and in the localized ones that reached within 22.5 deg of the speaker. As the second peak at the positive value of the

approach velocity was unique to the localized crickets, we calculated the boundary between these two distributions with different peaks from the data of all individuals using Otsu's method; this method is one of the popular algorithms used for image processing and can automatically allow us to identify the boundary which minimizes the sum of the weighted variances of the two groups (Otsu, 1979). The boundary value was 1.59 cm s^{-1}, which was used as the threshold to identify the starting points of the approach phase. When the crickets continuously moved towards the speaker at a higher approach velocity than this threshold, they were considered to be in the approach phase. Therefore, tracing the time
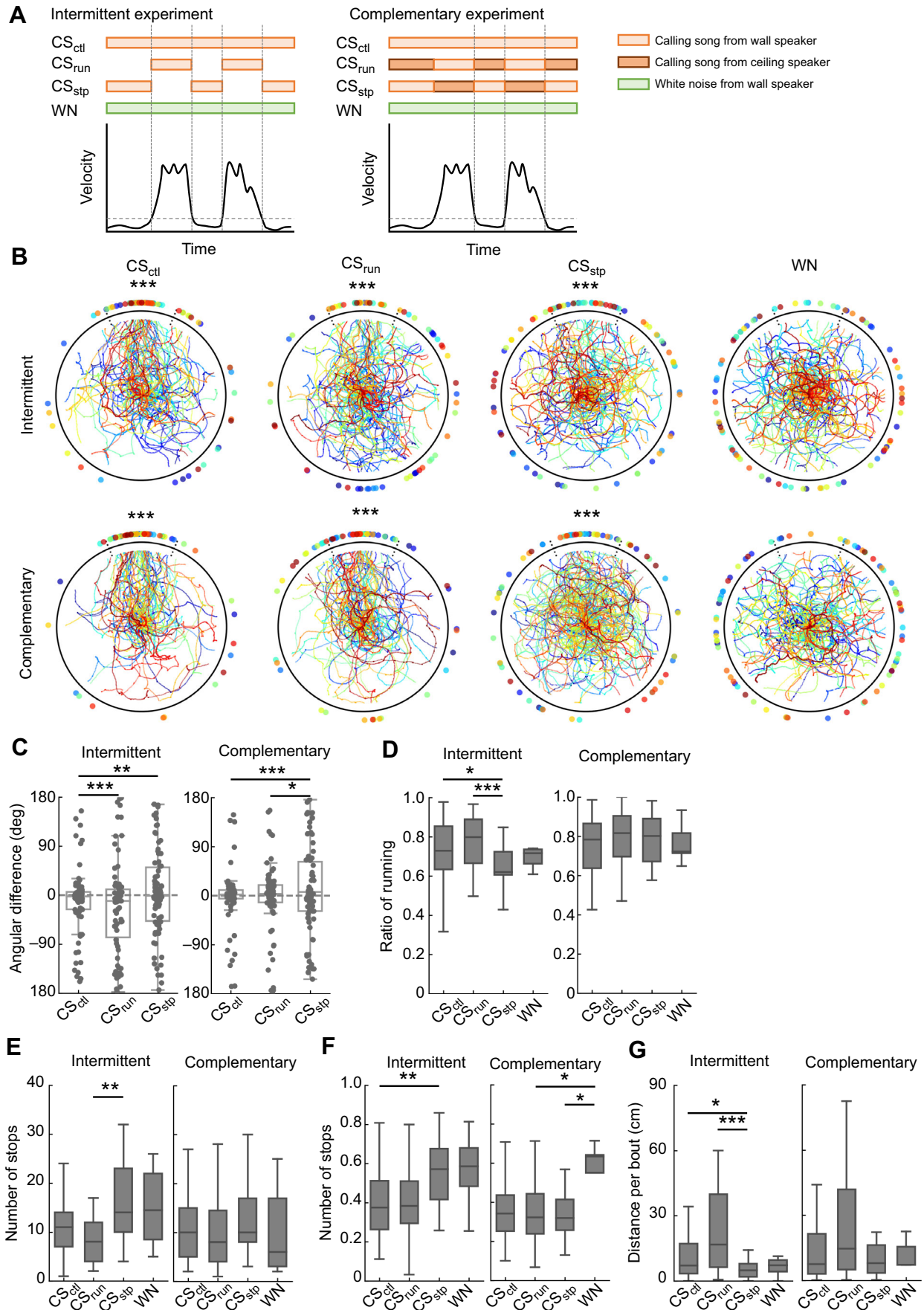
Fig. 5. See next page for legend.

Fig. 5. Effects of the closed-loop controlled intermittent sound presentation on phonotaxis. (A) Temporal scheme of the experiments using intermittent songs. In the intermittent experiment, the calling song was presented from the wall speaker only while running (CS_{run}) or stopping (CS_{stp}). In the complementary experiment, the sound source of the calling song was switched from the ceiling speaker to the wall speaker only while running (CS_{run}) or stopping (CS_{stp}). CS_{ctl} denotes control; WN, white noise. (B) Trajectories of individual crickets. The other descriptions are the same as in Fig. 2A. N=78 and 74 individuals for the intermittent and complementary experiments, respectively. (C) Angular differences between the arrival points and the wall speaker. Gray dots indicate the values for each individual. (D–G) Locomotor parameters of the localized crickets: (D) ratio of running period to total time from start to finish; (E) number of stops; (F) duration per stopping; (G) movement distance per running bout. N=48, 33, 29 and 8 individuals for CS_{ctl}, CS_{run}, CS_{stp} and WN conditions in the intermittent experiment, respectively; N=57, 43, 29 and 5 individuals for CS_{ctl}, CS_{run}, CS_{stp} and WN conditions in the complementary experiment, respectively. A V-test (B), Wallraff test with Bonferroni correction (C) and Wilcoxon rank sum test with Bonferroni correction (D–G) were used; significant differences: *P<0.05, **P<0.01, ***P<0.001.

course of the approach velocity back from the arrival time, the point at which the approach velocity was less than 1.59 cm s^{-1} for the first time, except for during a stopping period, was defined as the approach point (Fig. S2C). For this process, to remove high-frequency noise from temporal fluctuations in the approach velocity, the time series of the approach velocity changes was filtered by a median filter of 3 s.

Statistical analysis

All statistical tests were carried out with R programming software (version 3.3.2, https://www.r-project.org/). The statistical tests we used are described in the figure legends, and Table S1. When distribution of the data could be assumed as normal, we selected a parametric test; otherwise we used a non-parametric test. For the angular data, we used the circular R package (https://cran.r-project.org/web/packages/circular/). To test whether crickets were significantly localized around the speaker, a V-test, which is a modified Rayleigh test, was used. Here, 0 deg was specified as the mean direction indicating the sound source (Fig. 3A, top of the arena). In addition to the V-test, a Wallaff test was used to compare the distribution of the angular data between the different conditions.

RESULTS

Specific phase for directly approaching the sound source

Firstly, we examined how the sound level of the calling song affected the approach of the crickets to the sound source during phonotaxis. Movement trajectory was measured from the starting position at the center of the area to the finishing position where the crickets arrived at the arena wall (Fig. 1A). We applied the calling song or white noise at three different sound-pressure levels (60, 70 and 80 dB). When the calling song was playing, several crickets approached the sound source, and more crickets reached closer to the sound source when the sound pressure level was higher (Fig. 2). In addition, the crickets moved faster, and the ratio of running period to the whole duration was greater when the calling song was playing compared with when the white noise was playing (Fig. S3). These results indicate that cricket locomotive activity was enhanced when the calling song was louder (Fig. S3).

To investigate how crickets approached the sound source in response to the calling song, we focused on the individuals that were able to reach the speaker. For this purpose, we only analysed the trajectory of individuals that reached within 22.5 deg from the speaker (indicated by the two dotted lines in Fig. 2A). Under this

criterion, 17 out of 30 crickets stimulated by 80 dB songs were analysed. The trajectory of localized crickets showed two distinct phases. At first, the crickets wandered around the starting position, even when the calling song was playing, but suddenly approached the sound source from a specific point (Fig. 3A). The distance from the cricket to the speaker actually began to decrease from specific time points and was linearly reduced to the finishing point (Fig. 3B). This result indicated that the crickets approached the sound source in an almost straight trajectory from a specific time point. Thus, phonotaxis consists of two successive distinct phases: the first phase in which crickets wandered, and the second phase in which crickets approached the sound source.

To understand what triggered the transition between behavioral states in phonotaxis, the time point at which crickets started to move toward the sound source was defined. This point was referred to as the 'approach point' and was determined based on the velocity of crickets approaching the speaker. The approach points coincided with the starting point of rapid movement towards the speaker (Fig. 3B). It was possible that at the approach point the crickets happened to turn toward the sound source and locked onto the goal with sound inputs to both ears equally. To check this, the bearing of the speaker relative to the body axis of the crickets was measured (Fig. S4). If crickets faced the speaker, the bearing should be zero (Fig. S4A). However, the bearings at the approach points were not zero and became close to zero during the approach phase (Fig. S4B). Thus crickets might adjust their direction after starting to approach rather than locking onto the speaker before approaching. This result indicated that the orientation of crickets was not related to the start of the approach phase.

The spatial distribution of the approach points differed among the responses to calling songs at different intensities (Fig. 3C). For the 80 dB calling song, the approach points were close to the starting position, whereas approach points were scattered throughout the arena for the 60 and 70 dB songs. Therefore, the time delay from the starting point to the approach point for the 80 dB song was shorter than that for the 60 and 70 dB songs (Fig. 3D). Moreover, the sound pressure levels estimated at the approach point differed among the conditions (Fig. 3E). These results suggested that instantaneous SPL heard on the spot was not an external cue directly triggering a transition to the approach phase, but this transition could be facilitated by the louder calling song.

The large variance in the time delay from the starting point to approach points implied that temporal accumulation of hearing the calling song did not affect the transition much. To confirm this, we pre-exposed crickets kept in a cylinder to the calling song (CS_{ad}) or white noise (WN_{ad}) at the starting position from a speaker installed on the ceiling (Fig. S5A). We examined the effects of this pre-exposure on the phonotaxis induced by the calling song emitted from the wall-mounted speaker. The crickets exposed to WN_{ad} arrived in the area around the sound source, as did those exposed to CS_{ad} (Fig. S5B,E). Moreover, they showed the approach phase under both conditions (Fig. S5C,D). The location of the approach points with CS_{ad} and WN_{ad} were more scattered than those under control conditions (CS_{ctl}) without pre-exposure to auditory stimuli (Fig. S5F). Crickets exposed to CS_{ad} actually moved around the starting position (Fig. S5B,C). Therefore, the time delay from the starting point to approach points in CS_{ad} was longer than that in CS_{ctl}, indicating that the crickets needed a longer time to transition to the approach phase (Fig. S5G). These results demonstrated that the temporal accumulation of hearing the calling song did not facilitate phase transition. Rather, pre-exposure of the restrained cricket to an omnidirectional song delayed the start of the approach

phase. Taken together, although the transition to the approach phase could be affected by external stimuli, the start of the transition was not determined by instantaneous changes in the external environments. These results suggest that the behavioral transition was induced by changes in the animal's 'internal state'.

Maintenance of the approach phase requires a continuous calling song

Under the open-loop condition on the treadmill system, crickets moved towards the sound source by responding to the calling song. Thus they followed a simple rule of phonotaxis (Poulet and Hedwig, 2005). In contrast, our results under the closed-loop condition, such as with freely moving crickets, exhibited more complicated phonotaxis behavior with multiple phases, suggesting different rules (Fig. 3). In the approach phase, crickets appeared to approach the speaker straight, without wandering. One possible explanation for this was that the crickets memorized the sound orientation during the approach phase (Santos-Pata et al., 2018). We then examined whether crickets could approach the sound source even if the calling song disappeared during the approach phase. We changed the auditory stimulus or sound source location when the crickets moved 30 cm away from the starting position (Fig. 4A) because the approach points for the 80 dB song were distributed within 30 cm from the center (Fig. 3C). When the calling song was switched to white noise, both emitted from the wall speaker (CS_w–WN_w), the finishing points were distributed around the sound source (Fig. 4B). However, they were more scattered than those when the calling song was continuously played (CS_w–CS_w; Fig. 4C). This result suggested that the loss of spatial information and/or recognition of the calling song reduced the success rate of sound localization. To distinguish these two causes, we further tested the phonotaxis under the CS_w–CS_c condition in which the speaker emitting the calling song was changed from the arena wall to the ceiling (Fig. 4A). Under this condition, the crickets continued to recognize the calling song but lost the directional information of the sound source. The result under the CS_w–CS_c condition was similar to that under the CS_w–WN_w condition with the finishing points distributed around the sound source (Fig. 4B). As well as the CS_w–WN_w condition, the finishing points under the CS_w–CS_c condition were more scattered than those under the CS_w–CS_w condition (Fig. 4C). These results indicated that crickets did not memorize sound source orientation but needed to monitor the calling song continuously to reach the sound source.

Interestingly, we found some differences in the phonotaxis behavior between the CS_w–WN_w and CS_w–CS_c conditions. It took longer for crickets to arrive at the wall under the CS_w–WN_w condition than those under the CS_w–CS_w and WN_w–WN_w conditions, in which white noise was continuously emitted from the wall speaker (Fig. 4A,D). Under the CS_w–CS_w and CS_w–CS_c conditions, the ratio of running period increased after switching but decreased under the CS_w–WN_w condition (Fig. 4E). It suggested that this change was not the transition from the approach phase to the earlier phase. Moreover, the crickets under the CS_w–WN_w condition tended to stop more frequently after switching, unlike the other conditions (Fig. 4F). Taken together, the loss of calling song recognition but not of sound source orientation would downregulate the locomotive activity of the crickets. This implied phase transition to local search behavior.

Crickets detect sound direction while both running and standing still

The fact that the loss of the calling song induced the transition to the local search phase implied flexibility in cricket behavioral strategies depending on environmental conditions. To test this ability, we interrupted the calling song depending on cricket locomotion (the intermittent experiment; Fig. 5A). Even when the calling song was emitted only during running (CS_{run}) or standing still (CS_{stp}), the crickets could reach the area around the sound source (Fig. 5B, upper). This meant that crickets were able to approach the sound source regardless of the detection timing of the calling song. However, the success rate of sound localization under the CS_{run} and CS_{stp} conditions was lower than that in the positive control (CS_{ctl}) in which the song was continuously emitted (Fig. 5A). Moreover, the approaching process was different among the different conditions. The crickets localized in the CS_{stp} condition stopped more frequently and their running ratio was reduced (Fig. 5D,E). Remarkably, the duration of each stop was longer, and the running distance was shorter than those under the CS_{run} condition (Fig. 5F,G). This behavior of more frequent and longer stopping in the crickets that lost the calling song only while running may be advantageous for re-detecting the sound source location.

The intermittent experiment suggested that the transient loss of the calling song affected phonotaxis behavior depending on running and stopping. As shown in Fig. 4, the loss of song recognition reduced locomotion activity, which might result from the transition to the local search phase. This downregulation of activity might decrease the success rate of the CS_{run} and CS_{stp} conditions. To test this possibility, we emitted the calling song from the ceiling speaker to retain locomotion activity even when the calling song from the wall speaker was removed in the intermittent experiment (the complementary experiment; Fig. 5A). Similar to the intermittent experiment, crickets arrived at the area around the sound source (Fig. 5B, lower). However, the success rate of sound localization under the CS_{run} condition improved and was not significantly different from that under the control (CS_{ctl}) condition, in which the song was continuously emitted from the wall speaker (Fig. 5C). In contrast, the omnidirectional calling song emitted during running did not increase the success rate of sound localization under the CS_{stp} condition. These results suggest that the calling song heard while running would be more important for sound-source localization. For the complementary experiments, the approaching process did not differ in the rate of total running period, number of stops, running distance, or standing duration among the CS_{ctl}, CS_{run} and CS_{stp} conditions (Fig. 5D–G), indicating that the omnidirectional calling song could maintain the approach phase in crickets.

DISCUSSION

The trajectory of crickets stimulated by the calling song revealed two distinct behavioral phases that would not result from stereotypical behavior. While the conspecific calling song was playing, crickets initially wandered then started to directly move towards the sound source from specific time points. This result suggested that they did not reactively approach the sound source in phonotaxis. The approach points differed greatly in spatial and temporal location among individuals and were unrelated to *in situ* sound level or cumulative hearing experience. This meant that the transition to the approach phase was initiated based on the internal state of the crickets. Moreover, the retention of the approach phase required the recognition of the calling song, which was not necessarily a directed sound. When song recognition was lost, crickets stopped more frequently and reduced their mobility. These movements are typical for local search behaviors. Further experimentation suggested that crickets could alter their approaching behavior depending on their external environment. In

particular, it was more effective for sound-source localization to detect the calling song while running. These results indicate flexibility in the behavioral strategies of crickets.

In the present study, we showed that freely moving crickets in the arena approached the sound source via a specific behavioral phase referred to as the approach phase. Previous studies using a treadmill system showed that crickets responded to the short sound-pulse and exhibited a rapid steering behavior (Hedwig and Poulet, 2004; Poulet and Hedwig, 2005). This result supported the idea that a reactive motor response underlies cricket auditory behavior. Meanwhile, some research has explicitly suggested that cricket movement changed while they exhibited phonotaxis (Bailey and Thomson, 1977; Weber et al., 1981). Another study reported that the process of approaching the sound source in freely moving crickets was different between the initial and latter parts (Mhatre and Balakrishnan, 2007). Our study focused on the behavioral transition in this process and showed that crickets in the pre-approach phase did not exhibit a reactive response to the calling song. This suggests that the reactive motor response to the calling song may be specific to the later phase because the direct and straight movements during the approach phase seem to reflect the reactive response. In addition, the crickets in the approach phase reactively modulated their behavior, responding to the loss of song recognition. Thus, the auditory behaviors observed in the previous study might be specific to the approach phase we mentioned. The louder the calling song, the earlier the approach phase started (Fig. 3D). This might be because more auditory receptor cells were recruited and the central auditory neurons involved in phonotaxis such as AN1 and ON1 were more activated as the sound pressure level increased (Horseman and Huber, 1994; Lv et al., 2020), resulting in facilitation of the transition to the approach phase.

Our results indicate that phonotaxis is a complex process with multiple behavioral phases, including the approach phase. Sometimes animals do not respond immediately to changes in the external environment, but exhibit a specific behavior regardless of the timing of the stimulus. At this time, it is considered that their 'internal state' may be gradually shifted by the accumulation of sensory inputs and elicit that behavior. Here, the start of the approach phase would be decided by the internal state of the animal. Although the conditions of the calling song affected the start of the approach phase (Fig. 3D; Fig. S5G), the external environment did not deterministically cause the approach phase because the instantaneous SPL did not decide the timing of the phase transition (Fig. 3E). It is also possible that when crickets happen to face the sound source and the difference in SPL received by their two ears becomes smaller, crickets might stop wandering and start to approach the sound source (Rheinlaender and Blätgen, 1982). However, the bearing to the speaker was not related to the start of approach phase (Fig. S4). This fact revealed that the *in situ* external condition did not directly trigger the start of the approach phase. Rather, the phase transition would be caused by internal state change. The internal state could be affected by hormonal condition and temperature. For example, application of juvenile hormone modulating the internal state affects the choice of females during phonotaxis (Atkins et al., 2008; Koudele et al., 1987; Walikonis et al., 1991). Temperature could also affect the response in phonotaxis (Navia et al., 2015; Pires and Hoy, 1992). In the present study, however, there was a thermal effect only on the phonotaxis achievement when the SPL of the calling song was 60 dB (Fig. S6). Thus, the effect of temperature would be negligible for the approach phase in the phonotaxis induced by louder calling song.

Meanwhile, external changes in the auditory environment directly affected cricket movement in the approach phase. The loss of the song downregulated the locomotive activity and increased the frequency of stopping (Fig. 4). In addition, detecting the calling song while running was more effective for localization than while stopping. This might be due to differences in neural response to the calling song between running and standing still (Böhm and Schildberger, 1992; Schildberger and Hörner, 1988; Staudacher and Schildberger, 1998). However, when the crickets lost the calling song while running, such as under the CS_{stp} condition, the strong downregulation resulted in crickets stopping more frequently, resulting in a longer time to arrive at the sound source (the intermittent experiment; Fig. 5). In conclusion, during the approach phase the crickets may have an internal state that is more sensitive to the auditory environment, allowing them to respond to the calling song.

Moreover, we found that crickets could show another behavioral strategy, such as local search behavior, during phonotaxis. Behavioral changes in the local search depending on the external environment have been observed in various animal species (Corfas et al., 2019; Gray et al., 2005; Nevitt, 2008; Wolf et al., 2012). For example, restricting the search areas based on the surrounding olfactory cues associated with food can increase the success of foraging (Nevitt, 2000). For crickets, females have to search for their mating partner, relying on the calling song, which is intermittently interrupted by various obstructions in nature. The local search behavior would allow females to limit wasteful movement when the calling song is lost, and increase the chance of encountering neighboring males. Therefore, the behavioral change to the proper strategies in phonotaxis plays an important role in the reproduction of crickets. Detailed analysis of the trajectories in our study has revealed a more macroscopic perspective of phonotaxis.

Supplementary information
Supplementary information available online at
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