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Article

The best of both worlds: cicada males change costly signals to achieve mates while females choose a mate based on both calling and courtship songs

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Abstract

Cicadas usually sing and mate in the higher parts of trees. Studies addressing the effects of different acoustic signals on mate choice in Cicadidae are very limited. We investigated the effects of both acoustical features and morphological traits on mate choice in an East Asian cicada *Platypleura kaempferi*. Males produce high-rate calling songs that attract females, then produce low-rate courtship songs to secure mating when a female is attracted. Higher calling song rate (CR), shorter single-pulse duration, and shorter pulse period of the calling song, together with lower courtship song rate and longer echeme period of the courtship song, are the most desirable traits used by females to choose a mate. These traits indicate that the more a male can raise the rate of song production, the higher the probability he is sexually selected by the female. No correlation was found between morphological traits and mating success. After mating, a minority of males started emitting calling songs again, but the CR was significantly lower than before mating and none of them attracted a new mate later. This promotes females mating with unmated males. We hypothesize that *P. kaempferi* may have the best of both worlds due to the unique song modulation and the mechanism of female mate choice: males change energetically, costly acoustic signals to achieve mates, while females choose a mate based on males' acoustic properties. Our results contribute to better understanding the diversity of mating preference and enrich the mechanism of mate choice in acoustic insects.

Key words: acoustic insects, Cicadidae, courtship, mate choice, song modulation.

Choice in mating has been one of the central focuses of reproductive biology since Darwin (1871) introduced the concept of sexual selection. Mate choice generally has 2 main components: male–male competition and female choice (Darwin 1871). Male–male competition refers to any battle or competition among males for access to females and female choice describes selection by females of males according to male traits (Darwin 1871; Rosenthal 2017). Through active selection, a female may acquire direct resources (e.g., food or a nest site) from their chosen mates, and/or sperm that may contain higher-quality genes which may be passed on to their offspring (i.e., heritable benefits) (Brown 1999; Rosenthal 2017).

A large number of studies have been conducted on morphological traits related to mate choice in a variety of animal groups (e.g., insects, birds, frogs, fishes, and mammals) (Bateson 1983; Andersson 1994; Rosenthal 2017). In insects using sound communication, acoustic signals produced by males are particularly important for mate attraction and they are known to influence mate choice in a wide array of species, for example, crickets and cicadas (Alexander 1967; Doolan and Young 1989; Gerhardt and Huber 2002). Male insects usually produce acoustic signals with species-specific temporal and spectral features which allow females to recognize conspecific males (Ritchie et al. 1999; Nandi and Balakrishnan 2013). Variance in song parameters between males within a species may also allow conspecific females to discriminate between males (Doolan and Young 1989; Nishida and Takagi 2019). During pair-formation, some insects produce multiple types of acoustic signals. For example, males of most cricket species produce 2 types of acoustic signals, that is, the calling song to attract females from a distance and the courtship song during the short-range mating communication with females (Zuk et al. 2008). The variations of acoustic signals are suspected to indicate certain male attributes such as body size, age, nutritional status, or genetic condition, which may play a vital role in sexual selection (Wagner and Reiser 2000; von Helversen et al. 2001, 2004, 2015). Quantifying such variations in acoustic signals is one of the prerequisites for studying the mechanisms of mate choice in acoustic insects.

Cicadas (Hemiptera, Cicadoidea), well known for the loud songs produced by males, are among the most-studied insect groups in terms of sound production (Claridge 1985; Luo and Wei 2015). Previous studies have shown that males of some cicada species, as in many cricket species, can produce 2 types of acoustic signals during courtship: the calling song to attract females from a distance and the courtship song when a female is attracted by the male's calling song (viz., landing on the branch where the male is signaling) (Lei et al. 1994; Jiang et al. 1995; Chou et al. 1997; Cooley and Marshall 2001). Aside from calling songs, males of *Magicicada* species (e.g., Magicicada *septendecim* (L.) and Magicicada *cassini* (Fisher)) can also produce 2 types of courtship songs when they land in close proximity to females (Alexander and Moore 1958, 1962; Alexander 1967). Hou et al. (2017, 2021) found that in the rare Chinese cicada *Subpsaltria yangi* Chen, the males sometimes also emit sporadic courtship songs during the pair-formation process. In addition, Doolan and Young (1989) showed that in the bladder cicada *Cystosoma saundersii* (Westwood) identification of conspecific males by females is a 2-stage process, with the carrier frequency of the male calling song being more important in species recognition in long-range communication (flight of females to advertising males) and the temporal parameters of the calling song being more important in short-range communication (courtship). However, studies addressing the effects of different acoustic signals on mate choice in cicadas are very limited.

The East Asian cicada *Platypleura kaempferi* (Fabricius, 1794) is a common species widely distributed in China–Korea–Japan–Tsushima and the Ryukyu islands (Hou et al. 2014, 2018; Osozawa et al. 2017). The sounds made by *P. kaempferi* were briefly described in 2 previous studies (Hagiwara and Ogura 1960; Jiang et al. 1995). We investigated the acoustic behavior of *P. kaempferi* and analyzed the associations between mating success and 1) acoustic features of male song and 2) morphological characters of mated males. We used capture-mark-recapture/resight and morphometric analysis. We also analyzed the correlation between morphological characters and acoustic features of male song. The main aim of this study was to clarify the effects of the 2 distinct types of acoustic signals on mate choice in this species. This may provide further insight into mechanisms of mate choice in acoustic insects and other animals which produce vocal signals.

Materials and Methods

Study site and species

The study site is located at the Huolong Mountain (34°06′50.55″N, 108°02′40.02″E), Zhuyu Town, Zhouzhi County, Shaanxi Province, China. A natural population of *P. kaempferi* was found on the slopes at elevations between 680 and 690 m. The host plants of *P. kaempferi* in this habitat consisted of *Juglans regia* L., *Pyrus xerophila* Yü, *Armeniaca vulgaris* Lam., and *Cerasus yedoensis* (Matsum.) Yü et Li. The sampling arena covers a core area (~0.1 ha), where most calling activity occurred and a buffer zone (~10 m width) is adjacent to the core area. In the sampling arena, the host plant of *P. kaempferi* is mainly *C. yedoensis*, a widely used ornamental plant in China. The trees were 3.50 ± 0.32 m ($n = 21$) in height, which allows easy observation of the acoustic signaling and mating behavior of this cicada species in the wild.

Sampling, marking techniques, and morphological measurements

In our 2-year field surveys during 2018 and 2019, censuses of *P. kaempferi* were conducted using the capture-mark-recapture/resight method throughout the adult emergence of this species, that is, from 15 June to 9 August 2018 and 1 July to 9 August 2019. Newly emerged adults were caught at night after eclosion (Supplementary Figure S1). All individuals captured in the wild were measured using electronic Vernier calipers (with an error of ±0.01 mm) (Shengtai Electronic Technology Co. Ltd., China). We measured body length (BL), head width (HW), pronotum width (PW), mesonotum width (MW), and wing length (WL, right side). The timbal cover length (TCL) and timbal cover width (TCW), which may be related to the features of acoustic signals, were also measured. In the field survey in 2019, besides conducting morphological measurements, captured individuals were also weighed (within an error of ±0.001 g) with an electronic scale (Changxie Electronics Factory, Dongguan, China). Arabic numerals were then marked on both forewings of each individual with water-repellent red color. All marked cicadas were immediately released at the same location.

Capture-mark-recapture/resight technique and behavioral observations

The core area (~0.1 ha) was divided into 10 quadrats each 10 × 10 m. On each observation day (weather permitting), a systematic investigation of the area was conducted once every 4 h, from 09:00 to 17:00. This generally coincided with the period of sustained chorus activity of this species. To avoid any effect the time of day had on the activity of *P. kaempferi*, the first quadrat visited was rotated each day (in day 1 of sampling, we visited quadrats in the order of 1, 2, 3, 4, and 5; the following day's order was then 2, 3, 4, 5, 1, and so on). Remaining quadrats were then visited in ascending numerical order each day (Hou et al. 2021).

The behavior of this cicada species was observed under natural conditions and video-recorded using a Nikon Coolpix P100 digital camera (Nikon Corporation, Indonesia). Any effect on the cicadas was minimized by avoiding noise and sudden movement of the observer during behavioral observations.

In *P. kaempferi*, males produce calling songs (Supplementary Video S1) and sexually receptive females locate and fly to the advertising males based on their calling songs (see "Results"). In this study, any male that spent more than 30 min calling with no female approaching him was considered unsuccessful in attracting females. Once a female was successfully attracted by a male's calling songs, the male immediately changed its acoustic signals from the calling song to the courtship song, that is, he began to court the female (Supplementary Video S2) (see "Results"). Males that attracted a female by emitting calling songs but the female then flew away when the males emitted courtship songs (Supplementary Video S3) were considered unsuccessful in courting females. In our field investigation, a minority of successfully mated males started emitting calling songs again after mating. In this case, we observed their subsequent courting behavior after the mating until they flew away from our visual field (which was usually less than 30 min). Regardless of being successful or unsuccessful in attracting or courting females, the numerical code (identifier) of the male was noted.

When an individual was found unmarked in a mating pair during the surveys, we marked and measured/weighed it after the mating (if we had captured the unmarked individual), and then released it. Marked individuals that were never observed later were excluded from the analysis in this study. In total, 700 individuals (407 males, 293 females) were marked in the breeding season.

Song recording and acoustic analyses

All acoustic recordings were made using a linear PCM recorder with stereo microphones (PCM D100, Sony, China; frequency range 20–20,000 Hz and a 44.1-kHz/16-bit sampling resolution). All recordings were made at a distance of about 50 cm from the males. We recorded the acoustic signals of each male for approximately 3 min to ensure enough complete songs were recorded. Songs were recorded in WAV file format. The stereo recordings were converted to mono at a sampling rate of 44.1 kHz and 16-bit resolution. During song recording, the ambient temperature was measured using an electronic thermometer (Deli, Jiangsu, China), which ranged from 22°C to 39°C.

We successfully recorded the calling songs of 93 males who succeeded in attracting females. We also recorded the calling songs of 86 males who failed to attract a female. These calling songs were used to analyze female preference for characteristics of male calling song. We measured and compared 5 acoustic features of the calling songs: 1) calling song length (CL–parts 1–4 in Figure 1A–D), 2) calling song rate (CR), 3) single-pulse duration (PD) (Figure 1E), 4) pulse period (PP) (Figure 1E), and 5) dominant frequency (DF) (Figure 1F). As the third part of calling song (Figure 1A, C) is a long climax song, CR was calculated as the number of pulses per second based on Part 3 of the calling song. Ten complete calling songs of high-quality (i.e., possessing low background noise) were randomly selected from each male to measure the calling song properties, then mean values were calculated for subsequent analyses.

We successfully recorded courtship songs of 40 males who mated successfully. The courtship songs of 44 males who succeeded in attracting a female but failed in courting were also recorded. Courtship songs were used to analyze the female preference for the male's courtship songs. We measured and compared the following 5 acoustic features: 1) courtship song length (CSL) (Figure 2A, B), 2) courtship song rate (CSR), 3) single-echeme duration (ED) (Figure 2C), 4) echeme period (EP) (Figure 2C), and 5) DF of courtship song (CDF) (Figure 2D). CSR was calculated as the number of echemes per second. We also randomly selected 10 complete courtship songs of high-quality from each male to measure the courtship song properties, then mean values were calculated for subsequent analyses.

Acoustic analysis was conducted using Adobe Audition CC 2015 (Adobe, San Jose, CA) and Seewave (Sueur et al. 2008), a custom-made library using the R software platform (R Core Team 2011). Terms for acoustic signal description followed Wagner and Reiser (2000), Puissant and Sueur (2010), and Deb et al. (2012).

Statistical analyses

An index of body size for both sexes was generated through principal component analysis based on 4 morphological characters: BL, HW, PW, and MW. Correlations were performed between body weight and body size, between body weight and the ratio of WL/BL, and between body size and the ratio of WL/BL. Similarly, an index of timbal cover area of males was generated through principal component analysis based on TCL and TCW. Correlations were performed between the 10 acoustic features of male songs (including both calling and courtship songs). Correlations were also performed between the male song features and the following 3 morphological characters of males: BL, body size, and timbal cover area.

In our field investigation, male songs of *P. kaempferi* were recorded from 09:00 to 17:00, and there was a difference in ambient temperature between morning and afternoon. To clarify whether the ambient temperature affects the acoustic features of songs produced by the same individual, comparison of calling songs ($n = 14$ males) and courtship songs ($n = 7$ males) of the same male emitted in low ambient temperature in the morning and high ambient temperature in the afternoon was conducted based on paired-samples t-tests. In our field investigation, 9 males were found still advertising after mating. We recorded calling songs of 6 males both before and after mating and the differences in acoustic features of such individual's calling songs produced before and after mating were analyzed based on paired-samples t-tests ($n = 6$ males).

We used an independent-samples t-test to check the differences in acoustic features between different male groups, that is, males ($n = 93$) who succeeded in attracting females versus males ($n = 86$) who failed to attract a female; males ($n = 40$) who successfully mated versus males ($n = 44$) who failed in courtship. We then used logistic regression analysis to further reveal the relationship between acoustic features and mating success. According to the fitness-regression approach developed by Lande and Arnold (1983), we assigned an absolute fitness value of 1 to mating males and 0 to non-mating males.

We used 1-way ANOVA to check the differences in the 11 morphological characteristics (i.e., BL, WL, HW, PW, MW, TCL, TCW, body weight, body size, the ratio of WL/BL, and timbal cover area) among different male groups: 1) males who failed in attracting females ($n = 86$), 2) males who succeeded in attracting females but failed in courting females ($n = 63$), and 3) males who succeeded in attracting females and succeeded in courting females ($n = 54$). Then, it was followed by a Student–Newman–Keuls test, Tukey test, and a Bonferonni test.

Before the parameter test, we used Kolmogorov–Smirnov tests to determine whether data were distributed normally. The Levene test was used to test the homogeneity of the variance of the data. All statistical tests were 2-tailed and $P < 0.05$ was considered significant.

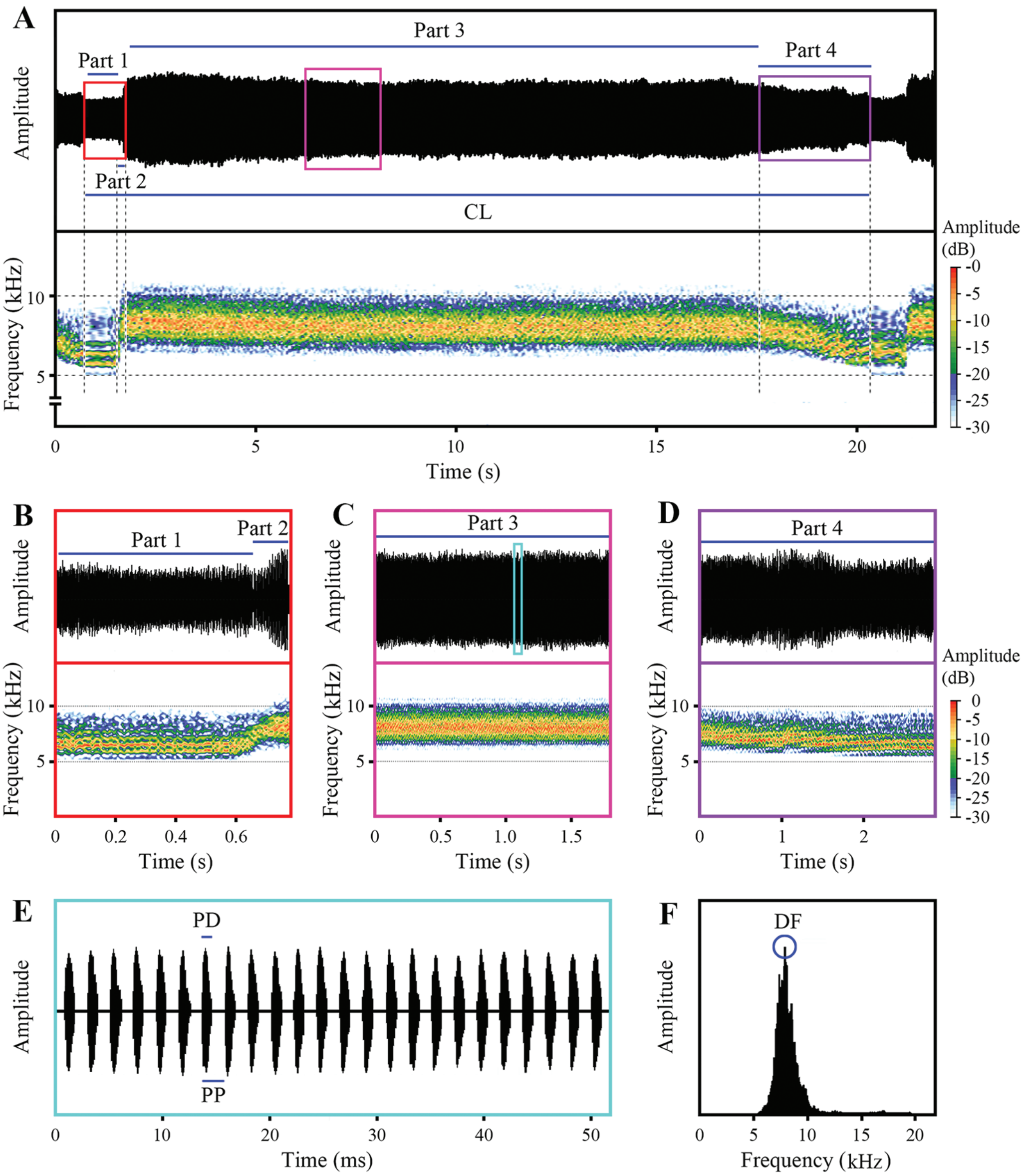

Figure 1. Acoustic analyses of the calling song structure of *P. kaempferi*. (**A**) Oscillogram (top half) and spectrogram (bottom half) of a calling song. (**B**) Oscillogram (top half) and spectrogram (bottom half) of Part 1 and Part 2 (marked by the red box in [A]). (**C**) Oscillogram (top half) and spectrogram (bottom half) of partial pulses in Part 3 (marked by the magenta box in [A]). (**D**) Oscillogram (top half) and spectrogram (bottom half) of Part 4 (marked by the purple box in [A]). (**E**) Detailed oscillogram of partial pulses in (C) (marked by the turquoise box in [C]). (**F**) Power spectrum showing the distribution of energy of the calling song, most of which is accumulated around a single frequency (DF).

Results

Courtship and mating behavior of *P. kaempferi*

In our 2-year field investigations, the total number of successful matings observed was 79 (24 in 2018, 55 in 2019). In addition, we recorded the calling songs of 179 males: of those 93 males succeeded in attracting females and 86 males failed to attract a female. We also recorded the courtship songs of 84 males after they had succeeded in attracting a female through their calling songs: 40 males succeeded in mating, while the other 44 males failed to secure a mate (Figure 3).

During pair-formation, males used their timbals to produce calling songs and courtship songs, which are distinctly different in structure (Figure 2A). Females relied on the calling songs to locate and find advertising males. When a male was signaling, the forewings were slightly opened, which was accompanied by the stretching vibration of the abdomen (Supplementary Video S1). Once a female was successfully attracted by a male's calling song (viz., flying around and finally landing on the branch where the male was signaling), the male immediately changed its acoustic signals from the calling song to the courtship song (Figure 2A). They then moved toward each other. The male produced courtship songs continually until physical contact occurred between the male and the female. When mating occurred, abdomens of the male and female connected with each other. Their bodies formed an angle at first, then moved into a straight line with the wings overlapping each other during mating (Supplementary Video S2). The mating duration was 35.33 ± 5.88 min (mean ± SD [standard deviation], $n = 48$ pairs) (we only included data on duration where the complete mating sequence was observed: initiation of pairing to separation). The female flew away when the mating was over. The male either remained

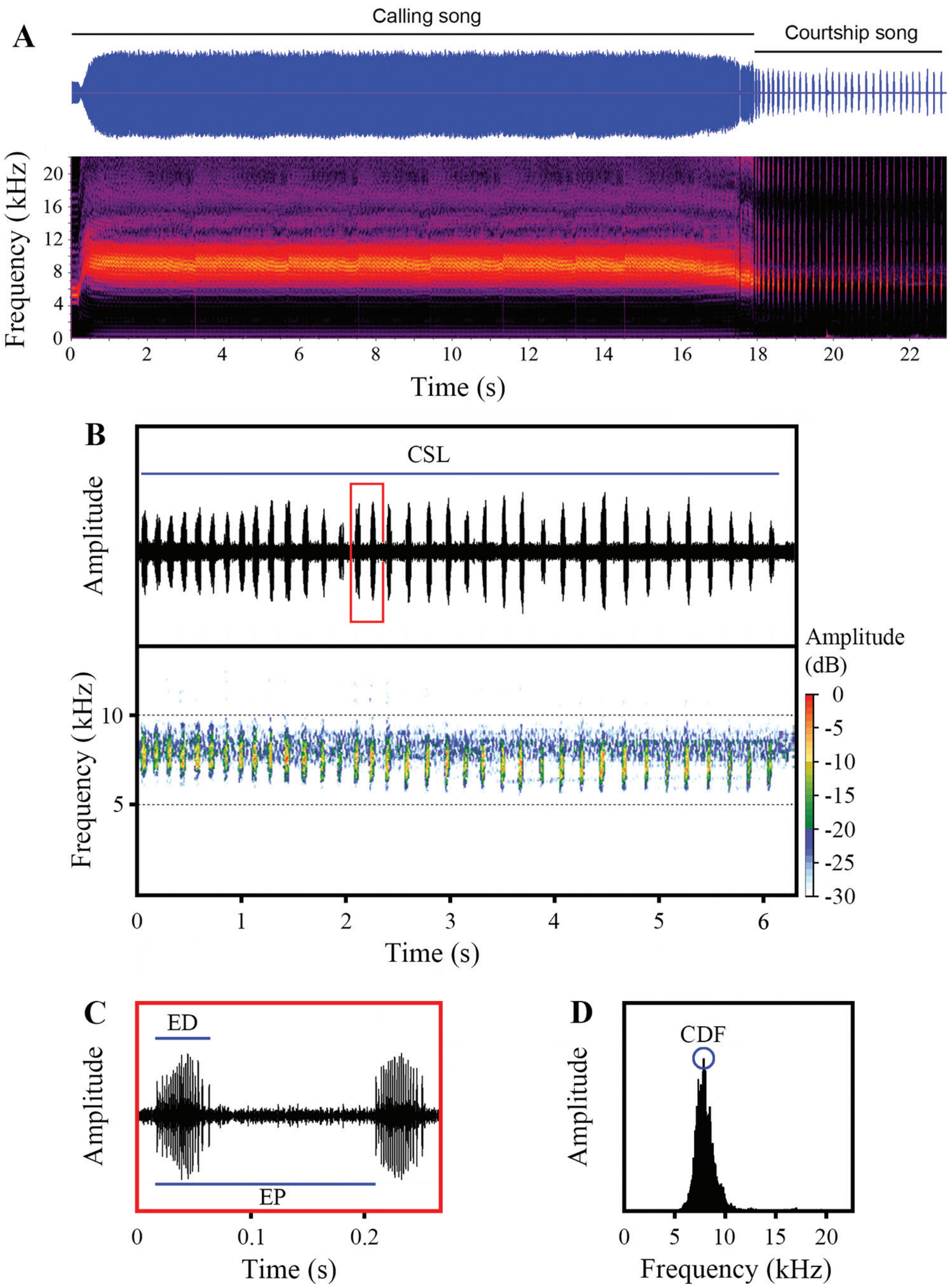

Figure 2. Acoustic analyses of the courtship song structure of *P. kaempferi*. (**A**) A male changing its acoustic signals from the calling song to the courtship song. Top half: oscillogram, bottom half: spectrogram. (**B**) Oscillogram (top half) and spectrogram (bottom half) of courtship song. (**C**) Detailed oscillogram of partial echemes in (B) (marked by the red box in (B)). (**D**) Power spectrum showing the distribution of energy of the courtship song, most of which is accumulated around a single frequency (CDF).

on his perch after copulation, or flew away after the female left. However, 9 mated males started to emit calling songs again immediately after mating, but none of them attracted a second female during our subsequent field investigations.

We observed many times where 49 males (in which we recorded courtship songs of 44 males) were rejected by a female who had been successfully attracted by the male. In this case, although the male scratched the female's wings with his forelegs and continuously emitted courtship songs, the female flapped her wings to knock off the male and then flew away (Supplementary Video S3). Once rejected, the male began emitting calling songs again.

The effect of temperature on male songs

In our field investigations, we recorded calling songs of 14 males both in the morning with lower ambient temperature (25–30°C) and in the afternoon with higher ambient temperature (29–38°C). A paired-samples *t*-test analysis revealed that there were no differences in the 5 acoustic features of calling songs produced by the same male (Table 1). We also recorded courtship songs of 7 males both in the morning with lower ambient temperature (26–29°C) and in the afternoon with higher ambient temperature (31–38°C). The paired-samples *t*-test analyses revealed that there were no differences in the 5 acoustic features of courtship songs produced by the same male (Table 1).

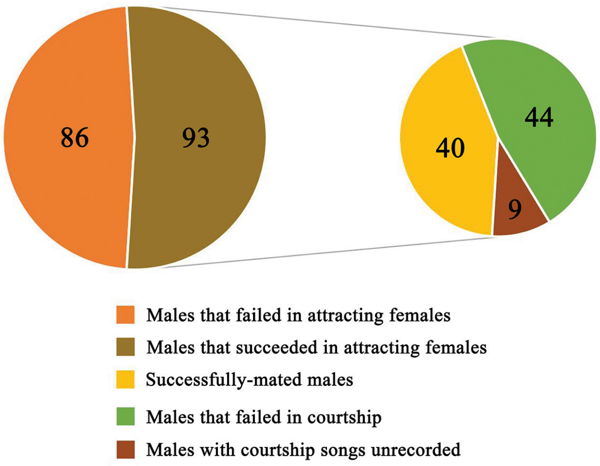

Figure 3. Statistics of males attracting versus not attracting females and then mated versus non-mated with females.

Song structure of *P. kaempferi*

A complete calling song of *P. kaempferi* can be divided into 4 parts (Figure 1A) with a total duration of about 18.049 ± 3.126 s (mean ± *SD*, $n = 179$; Table 2). The first part (Figure 1A, B), lasting approximately 0.604 ± 0.133 s, is a start song. The second part (Figure 1A, B) is a very short acceleration song, with a duration of 0.152 ± 0.048 s. The third part (Figure 1A, C) is a long climax song with a duration of approximately 14.721 ± 3.000 s, which is formed by a series of pulses (Figure 1E) with each pulse having a duration of 1.033 ± 0.112 ms (Table 2). The fourth part (Figure 1A, D) is a short deceleration song, with a duration of 2.666 ± 0.158 s. The DF (Figure 1F) of the calling song is 8.211 ± 0.242 kHz (Table 2). Pearson correlation coefficients between calling song properties indicate that males calling at a higher rate had shorter PD and shorter PP than those calling at a lower rate. The 4 temporal features (CL, CR, PD, and PP) are not correlated with the DF (Supplementary Table S1).

A complete courtship song, with a duration of 6.558 ± 1.155 s (mean ± *SD*, $n = 84$; Table 2), is formed by a series of echemes (Figure 2A, B). Each echeme (Figure 2C) has a duration of 46.725 ± 4.866 ms (Table 2). The DF (Figure 2D) of the courtship song (CDF) is 8.222 ± 0.268 kHz (Table 2). Pearson correlation coefficients between courtship song properties indicate that males courting at a higher rate had shorter ED and shorter EP than those calling at a lower rate. The 4 temporal features (CSL, CSR, ED, and EP) are not correlated with the spectral property (CDF) (Supplementary Table S1). The DF of a courtship song is highly positively correlated with the DF of a calling song within individuals (Supplementary Table S1).

Morphological characteristics and their correlations with acoustic features

The first principal component for the males explains 73.863% of the variance and is used as a measure of overall body size (Supplementary Table S2). For males, body size $\text{PC1} = 0.247 \times \text{BL} + 0.312 \times \text{HW} + 0.301 \times \text{PW} + 0.300 \times \text{MW}$. The first principal component for the females explains 77.656% of the variance and is used as a measure of overall body size (Supplementary Table S2). For females, body size $\text{PC1} = 0.260 \times \text{BL} + 0.299 \times \text{HW} + 0.289 \times \text{PW} + 0.285 \times \text{MW}$.

Male body weight and male body size are positively correlated ($r = 0.741$, $P < 0.001$, $n = 165$) (Supplementary Figure S2A). Female body weight and female body size are also positively correlated ($r = 0.689$, $P < 0.001$, $n = 108$) (Supplementary Figure S2B). The WL of males is longer than their BL (Supplementary Figure S2C). Male body weight is not correlated with the ratio of WL/BL ($r = -0.111$, $P = 0.157$, $n = 165$) (Supplementary Figure S2C). The WL of females is also longer than their BL (Supplementary Figure S2D). Female body weight is also not correlated with the ratio of WL/BL ($r = -0.096$, $P = 0.323$, $n = 108$) (Supplementary Figure S2D). These results indicate that there is no significant correlation between body weight and flight ability in both sexes.

Male body size and the ratio of WL/BL are negatively correlated ($r = -0.416$, $P < 0.001$, $n = 165$) (Supplementary Figure S2E). Female body size and the ratio of WL/BL are also negatively correlated ($r = -0.259$, $P = 0.007$, $n = 108$) (Supplementary Figure S2F). These findings indicate that the larger the body size, the smaller the ratio of WL/BL, which may suggest individuals with a larger body size have a weaker flight ability.

Pearson correlation analysis was performed between the 10 acoustic features (CL, CR, PD, PP, DF, CSL, CSR, ED, EP, and CDF) and 3 morphological characteristics (i.e., BL, body size PC1, and timbal cover area). Neither the temporal features nor the DF were found to be correlated with any morphological characteristics (Supplementary Table S3).

Table 1. Comparison of calling songs and courtship songs produced by the same male in lower and higher ambient temperatures based on paired-samples *t*-tests

Calling song ($n = 14$ males)					Courtship song ($n = 7$ males)				
Acoustic features	$\Delta\bar{x}$	*SD*	t_{13}	*P*	Acoustic features	$\Delta\bar{x}$	*SD*	t_6	*P*
CL	–0.159	2.016	–0.296	0.772	CSL	–0.447	1.445	–0.818	0.445
CR	12.062	23.231	1.943	0.074	CSR	0.503	1.589	0.838	0.434
PD	–0.008	0.141	–0.210	0.837	ED	–0.088	2.637	–0.088	0.933
PP	0.041	0.156	0.980	0.345	EP	–17.022	40.246	–1.119	0.306
DF	–0.107	0.313	–1.280	0.223	CDF	–0.076	0.317	–0.632	0.551

Note: $\Delta\bar{x}$ = mean (lower temperature) – mean (higher temperature); SD, standard deviation; *t*, *t*-values for the paired-samples *t*-tests; *P*, *P*-values for the paired-samples *t*-tests.

Table 2. Description of the acoustic features of male songs

Calling song ($n = 179$ males)		Courtship song ($n = 84$ males)	
Acoustic features	Mean ± *SD*	Acoustic features	Mean ± *SD*
CL (s)	18.049 ± 3.126	CSL (s)	6.558 ± 1.155
CR (per s)	484.942 ± 38.977	CSR (per s)	7.895 ± 1.296
PD (ms)	1.033 ± 0.112	ED (ms)	46.725 ± 4.866
PP (ms)	2.104 ± 0.145	EP (ms)	136.116 ± 12.872
DF (kHz)	8.211 ± 0.242	CDF (kHz)	8.222 ± 0.268

Note: SD, standard deviation.

Correlations between acoustic features and mating success

We found significant associations between mating success rate and 3 temporal properties of the calling song (Table 3). The higher the CR (Figure 4A), the shorter the PD (Figure 4B) and the shorter the PP (Figure 4C), the higher the mating success rate of males. Analysis of the relationship between the mating success rate and the acoustic features of courtship songs (Table 4) revealed that the lower the CSR (Figure 4D) and the longer the EP (Figure 4E), the higher the mating success rate of males.

For males that started emitting calling songs to attract females after mating, the paired-samples *t*-test analyses revealed that the CR was significantly lower and the PD and PP were longer than before mating ($n = 6$) (Table 5). Although they restarted calling songs after mating, none of these individuals succeeded in attracting a second female during our subsequent field investigations.

Comparison of morphological characters in males

The first principal component for the males explains 75.130% of the variance in timbal cover area. For males, timbal cover area $\mathrm{PC1} = 0.577 \times \mathrm{TCL} + 0.577 \times \mathrm{TCW}$. Results of 1-way ANOVA show that no significant differences exist in the morphological characters (including BL, WL, HW, PW, MW, TCL, TCW, body weight, body size, the ratio of WL/BL, and timbal cover area) among males that failed in attracting females, males that succeeded in attracting females but failed in courting females, and males that succeeded in both attracting and courting females (Supplementary Figure S3).

Discussion

Cicadas usually perch, sing, and mate on the higher parts of their host plants. This makes the observation of courtship and mating behavior of cicadas very difficult. Thus, few studies have been conducted on cicadas to address the role of different acoustic signals on mate choice. *Cerasus yedoensis* trees, the host plants of *P. kaempferi* at our study site, were about 3–4 m in height when we conducted the field observations. This allowed relatively easy observation of the behavior of this cicada species. Results of our study confirm that males of *P. kaempferi* produce 2 types of acoustic signals during pair-formation: the calling song (long-range communication) used to attract females, and the courtship song (short-range communication) used to achieve mating. The behavior of males producing these 2 types of acoustic signals was also found in the periodical cicadas (*Magicicada* spp.) (Cooley and Marshall 2001). We reveal a unique song modulation in *P. kaempferi*: males producing high-rate calling songs but low-rate courtship songs, and the calling song coupled with the courtship song playing roles in pair-formation and female mate choice. In our field investigation, songs of *P. kaempferi* males were recorded from 09:00 to 17:00, with higher ambient temperatures in the afternoon, but our analyses confirm that there is no difference in the 10 acoustic features of the songs produced by the same male at different ambient temperatures. Chatfield-Taylor and Cole (2019) analyzed 227 recordings belonging to 23 taxa of cicadas and found that neither syllable rate nor peak frequency was correlated with temperature. Our result is consistent with Chatfield-Taylor and Cole (2019), although the sample size of our study is limited, suggesting that all of the acoustic features could be compared without considering the effect of temperature.

By making their signals stand out, males can make themselves more detectable and easier to locate (Ryan and Cummings 2005; Wilson and Mennill 2011; Mowles and Ord 2012). Calls may play an important role in mate choice by acting as indicators of male quality (Alexander 1967), since call-production is a highly energetically demanding activity (Prestwich and Walker 1981; Taigen and Wells 1985; Gerhardt and Huber 2002). Studies of mate choice in acoustic insects have shown that female preferences for male acoustic features are focused on all features requiring larger energy expenditure, such as calling for longer and louder, or calling at higher rates (Doubell et al. 2017; Erregger et al. 2017). In our study, we show that males of *P. kaempferi* who succeeded in mating have a significantly higher CR than those that failed to attract females. A similar phenomenon was found in the cicada *S. yangi*, in which males who produced calling songs with a higher rate achieved greater mating success (Hou et al. 2021). Males of such species emitting calls at a higher rate may increase a male's probability of obtaining females and reduce the females' search costs (Fellers 1979; Sanches et al. 2017). In contrast, *P. kaempferi* males that produce courtship songs at a relatively lower rate are more likely to be sexually accepted by females. Gwynne (1987) revealed that in the tick-tock cicada *Physeema quadricincta* (Walker), males produce courtship songs at a lower rate in comparison to the relatively high-rate calling songs, but the effect of the 2 types of acoustic signals in mate choice has never been investigated. In the bladder cicada *C. saundersii*, the carrier frequency of the male calling song is of more importance in species recognition in long-range communication (flight of females to advertising males), while the temporal parameters of the calling song being more important in short-range communication

Table 3. Comparison of the calling song features between males that succeeded or failed in attracting females

Acoustic features of calling song	Males that succeeded in attracting females ($n = 93$ males)	Males that failed in attracting females ($n = 86$ males)
CL	18.023 ± 3.248^{a}	18.077 ± 3.007^{a}
CR	510.214 ± 29.228^{a}	457.614 ± 28.325^{b}
PD	0.969 ± 0.066^{b}	1.103 ± 0.110^{a}
PP	2.008 ± 0.077^{b}	2.207 ± 0.130^{a}
DF	8.237 ± 0.260^{a}	8.183 ± 0.220^{a}

Note: Different letters represent significant difference at the 0.05 level.

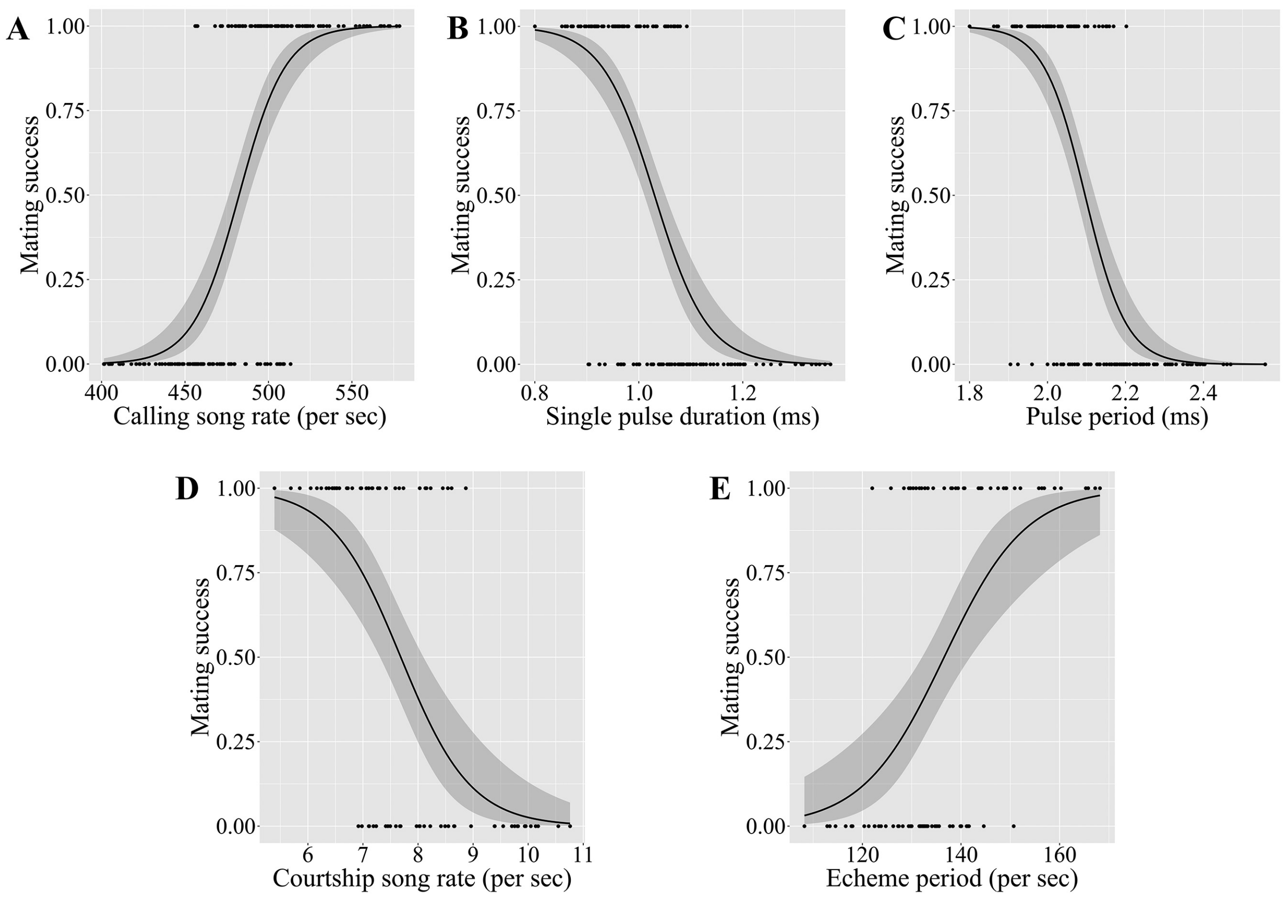

Figure 4. Correlations between male mating success and 5 acoustic features. (**A**) Correlation between male mating success and CR. (**B**) Correlation between male mating success and PD. (**C**) Correlation between male mating success and PP. (**D**) Correlation between male mating success and CSR. (**E**) Correlation between male mating success and EP.

Table 4. Comparison of the courtship song features between successfully mated males and males that failed in courtship

Acoustic features of courtship song	Successfully mated males ($n = 40$ males)	Males that failed in courtship ($n = 44$ males)
CSL	6.382 ± 1.156^{a}	6.719 ± 1.144^{a}
CSR	7.062 ± 0.846^{b}	8.653 ± 1.167^{a}
ED	47.073 ± 5.250^{a}	46.409 ± 4.526^{a}
EP	143.206 ± 12.508^{a}	129.671 ± 9.417^{b}
CDF	8.236 ± 0.261^{a}	8.209 ± 0.277^{a}

Note: Different letters represent significant difference at the 0.05 level.

Table 5. Comparison of calling songs produced by the same male before and after mating based on paired-samples *t*-tests ($n = 6$ males)

Acoustic features	Before mating	After mating	t_5	*P*
CL	16.598 ± 2.478	17.141 ± 1.083	−0.666	0.535
CR	510.837 ± 18.582	443.879 ± 35.572	5.316	0.003
PD	0.978 ± 0.063	1.260 ± 0.193	−3.674	0.014
PP	1.967 ± 0.072	2.318 ± 0.211	−4.199	0.008
DF	8.197 ± 0.351	8.032 ± 0.234	2.031	0.098

Data are given as means ± SD (standard deviation).

(courtship) (Doolan and Young 1989). Results of our study argue that *P. kaempferi* females prefer to select male producing calling songs with a higher rate and courtship songs with a lower rate.

Previous studies have shown that signaling at a higher rate in some species can act as a handicap for males to obtain a mate, because it is energetically costly (Alexander 1967; Gerhardt and Huber 2002; Ryan and Cummings 2005; Mowles and Ord 2012). For males, there may be a trade-off between call effort and other effort(s) (e.g., sperm production) (Wedell et al. 2002; Rosenthal 2017). If it is the case that *P. kaempferi* females show a preference for both higher-rate calling song and higher-rate courtship song, the energetically costly sound-production might make males become weaker, which may decrease their mating ability or result in lower sperm production (Wedell et al. 2002; Rosenthal 2017). Given that the higher CR coupled with the lower CSR are the most desirable traits for *P. kaempferi* females to choose a mate, we infer that producing a courtship song at a higher rate may be a handicap for *P. kaempferi* males in obtaining a mate, while the females can choose a better mate (i.e., a male with stronger ability to change the rate of songs) for their offspring according to the males' acoustic properties.

Previous studies have shown that male cicadas may mate multiple times but females mate only once due to males possibly using mating plugs to prevent re-mating of females (White 1973; Karban 1983; Dougherty et al. 2016). In our field observation, we found that a minority of males ($n = 9$) started emitting calling songs after mating (which might be due to a copulation being interrupted by a predator or some other reason), but they were not successful in attracting a second mate during our subsequent observation. Although the sample size is limited and perhaps the window of recovery of such males was missed, we found that the CR of such individuals had significantly decreased after mating. This provides direct evidence to support the suggestion that mating is an energetically demanding activity and may reduce the likelihood of males mating again, and suggests that the mechanism of female mate choice in this species may promote females mating with unmated males. Future work might consider how mating and re-mating influence the acoustic properties and mate choice in other acoustic insects, which may be informative for studies of mate choice evolution in acoustic insects.

Morphological characters of insects are often the most intuitive traits and many insects incorporate morphological characters in their criteria for mate choice. For example, a previous study on periodical cicadas revealed that females of *M. cassini* prefer larger males (Karban 1983). Mating males were found to be statistically smaller than chorusing males in another periodical cicada species, *M. septendecim* (Cooley and Marshall 2004). However, morphological traits of *P. kaempferi* males are not correlated with the mating success rate. We have shown that the body size of males is significantly, negatively correlated with the ratio of WL/BL in *P. kaempferi*, which indicates that the larger the body size, the weaker the flight ability of males. In addition, no significant differences in morphological traits were found among males that failed to attract females, males that succeeded in attracting females but failed in courting females, and males that succeeded in both attracting and courting females. These results indicate that a female preference for male morphological traits might not evolve in *P. kaempferi*.

Previous studies have shown that the variation in acoustic signals between individuals may be caused by internal factors such as the body weight, size, and energy state (Latimer and Schatral 1986; Römer and Bailey 1986; Anichini et al. 2018). A high-quality signal can convey an individual's superior body condition, which may be related to male–male competition and female mate choice (Wilgers and Hebets 2015). For example, heavy males of the bushcricket *Poecilimon ampliatus* (Brunner von Wattenwyl) produce longer verses and higher duty cycles when competing with light competitors (Anichini et al. 2018). Studies of the tree cricket *Oecanthus henryi* (Chopard) revealed that not only the chirp period but also the syllable period as well as the syllable duration were positively correlated with the body size of males (Deb et al. 2012). However, no correlations were found between acoustic features and morphological characters in *P. kaempferi* males. This suggests that acoustic features might only be associated with the structure of sound-producing organs (i.e., the timbals) in cicadas, which does not convey information on body mass.

In conclusion, this study revealed that it is the calling song coupled with the courtship song, rather than morphological traits, that play important roles in female mate choice in the cicada *P. kaempferi*. We revealed that males produce high-rate calling songs first to attract females, then change the calling song to a low-rate courtship song when a female is attracted. We further revealed that the higher CR, shorter PD, and shorter PP of calling songs, as well as the lower CSR and longer EP of courtship songs, are the most desirable traits for females to choose a mate. We hypothesize that *P. kaempferi* may have the best of both worlds: males change acoustic signals to achieve mates and females choose a better mate for their offspring from numerous males based on males' acoustic properties. Results of our study contribute to better understanding the diversity of signaling behavior and mating preference in acoustic insects, which enrich the mechanisms of mate choice in animals.

Authors' Contributions

C.W. conceived the study. S.W. and Z.H. collected and analyzed the data. All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

Conflict of Interest

The authors declare that they have no conflict of interest.

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