Variations in male calls and responses to an unfamiliar advertisement call in a territorial breeding anuran, *Rana dalmatina*: evidence for a "dear enemy" effect

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Focusing on the male advertisement call in *Rana dalmatina*, this study addresses two issues: (i) there is a large variability in call characteristics among males (fundamental frequency range: 571-865 Hz, number of pulses: 8-87) and (ii) males are able to discriminate between neighbouring and unfamiliar conspecifics. In response to an unfamiliar acoustic stimulus, call advertisement presented the longest duration. In contrast, the response to a familiar conspecific call did not show any significant difference from solitary vocalisations. Emphasising the existence of a discrimination, this result may be regarded as a "dear enemy". This decremental reply may result from habituation to a neighbour's call, constituting a learning process. Thus, we suggest call familiarity may enhance conspecific tolerance, reducing the expensive energetic cost of defence interactions for this territorial breeding species.

KEY WORDS: call, variability, tolerance, Agile frog.

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INTRODUCTION

In many species of anurans, mating calls occur as mechanisms of both specific pre-zygotic identification and sexual selection. Males emit calls as attractive signals and advertisement patterns could be selected through female choice (GERHARDT 1994, MURPHY & GERHARDT 1996, LODÉ 2001). The sexual selection theory predicts that male anurans produce individually distinct calls because variations in male call patterns could result in better reproductive success (GERHARDT & DAVIS 1988, RYAN & WILCZYNSKI 1991, RYAN & KEDDY-HECTOR 1992, COCROFT & RYAN 1995). However, due to the peculiarities of the vocal apparatus, only some properties of the sound, mainly call rate and duration, may vary depending on the species considered (GERHARDT & WATSON 1995). Both field observations and experimental studies emphasise that females are attracted by loud and long calls mainly produced by the oldest and most experienced animals (HOWARD & YOUNG 1998). As the male call is likely to direct female choice, natural selection is also expected to reduce call variability and to stabilise advertisement call patterns in anurans.

Acoustic communication is a main fitness related trait. Because of male-male competition for mating, males have to perform a call that is more attractive than that of their conspecifics (ARNTZEN 1999). This strong competition in calling activity leads males to seize a calling site and this territorial breeding behaviour reduces direct competitive interactions (BRZOSKA 1982, COCROFT & RYAN 1995, BURMEISTER et al. 1999). Males are able to discriminate among potential calling sites in regard to the absence of predator and site value (RESETARITS & WILBUR 1991). In this competitive situation, the tolerance of a conspecific may be a good strategy for any male, as a trade off with a costly territoriality. The processes for recognition of the neighbour is based on acoustic discrimination (DAVIS 1987). In fact, being able to settle side by side in good breeding sites, males could recognise and bear each other. If frog's response to the calls differs depending on whether the signal is familiar or unfamiliar, it could be assumed that males more actively respond to any emission of an unfamiliar signal. Hence, such discrimination may be regarded as a cognitive process and then, it could be predicted that this process plays an important role in increasing conspecific tolerance in *Rana dalmatina*.

The Agile frog *Rana dalmatina* is extremely widespread in Europe but it is not as common as other species and breeding congregations are rarely numerous (GEISSELMANN et al. 1971). Thus, the main purpose of this study was (i) to identity the variables involved in mate choice and (ii) to record reactions to familiar male advertisement calls compared with the reactions to unfamiliar conspecific advertisement calls, assuming that competition could be involved in the call variability. Thus, this study provides new evidence on the role of neighbour tolerance recognition and on the effect of unfamiliar vocalisation on a territorial breeding frog.

MATERIAL AND METHODS

Subjects

Calling activity of 67 males of *Rana dalmatina* was studied during the breeding period between 22:30 hr and 24:00 hr, local time, in February and March, from 1994 to 2000. In

these periods, the air temperature was 4-14 °C and the water temperature 6-10 °C. Sampling was realised in 15 small permanent breeding ponds (range 45-212 m^2), in western France, within 30 km of Angers. In each pond, the number of individuals range from 4 to 17 calling males (mean = 9.3, SD = 3.7, n = 15).

Apparatus

Individual advertisement calls were tape-recorded using a microphone (Lem Communication 4535 electret with EM 700 condenser shot gun, 20 to 22000 Hz at 8 dB, 600 Ω, sensibility 3-27 dB, efficacy – 69 dB, distortion < 0.3%) and a Digital Audio Tape Recorder (Sony DAT TCD-D8, sampling frequency 44.1 kHz, 20 Hz-20 kHz, 16 bit resolution).

Rana dalmatina calling characteristics

The Agile frog presented two types of call and are able to call under water (SCHNEIDER et al. 1988). We only focused on calls emitted at the surface. Calls were sampled for 1 min in order to determine oscillograms and spectrograms. They were analysed with two different software programs using Mathematic Fast Fourier Transformation (resolution 22 Hz, 1024 points), Spectrogram 4.2 (HORNE 1994) and Avisoft saslab pro version (SPECHT 1998). Calls were analysed for four different acoustic parameters: number of pulse groups per call (n), call duration (msec), duration/n pulse groups and fundamental frequency (Hz). A coefficient of variation as the ratio "standard deviation/mean" (GERHARDT 1991) was used to estimate parameters which displayed a high variation.

Response to familiar and unfamiliar calls

A sample of 22 males were recorded because they each one clearly replied to both familiar and unfamiliar calls. The absence of significant differences between their call characteristics and the other males characteristics was tested using the Welch's approximate t-test. Individual calls were recorded in field conditions reflecting natural competitive interactions during the breeding period. Play back calls were emitted for 20-25 sec corresponding to 3 calls in the immediate vicinity (range 1-3 m) of a male calling. Two kinds of play back were emitted at a sound pressure level lower than the original by about 3-10 dB (BRZOSKA et al. 1982): familiar (i.e. play back of a call from a male of the same pond) an unfamiliar (i.e. play back of a call from a male of a different pond). The reaction was immediately recorded using the DAT recorder. The test was stopped as soon as a male reply was recorded for 1 min. Only three tests were performed when the male did not react within 5 sec, considering that later call may not be defined as an 'answer' (BRZOSKA 1982, BRZOSKA et al. 1982). We assessed variation in the same calling parameters as mentioned above in order to specify if modifications arose in the answer. The responses of males were recorded during the same night whether they were confronted with familiar or unfamiliar recorded advertisement calls. Comparisons between acoustic parameters were made using Repeated Measures Analyses of Variances and the Student-Newman-Keuls multiple comparisons test.

<div align="center">RESULTS</div>

Rana dalmatina calling characteristics

The call of *Rana dalmatina* was composed of a succession of pulse groups repeated several times (Fig. 1). Each pulse group consisted of 3 to 12 pulses
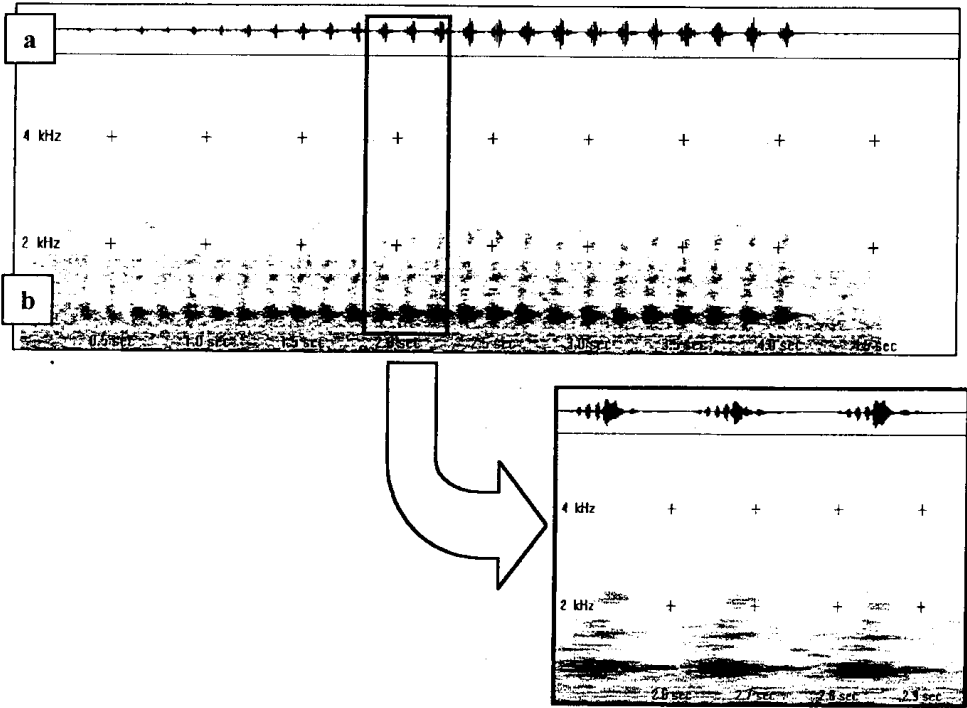
Fig. 1. — Oscillogram (a) and spectrogram (b) of a male *Rana dalmatina* advertisement call (n = 67).

increasing in volume (see GEISSELMANN et al. 1971 and SCHNEIDER et al. 1988 for details on this sub-structure). The advertisement call duration ranging from 1416 msec to 13793 msec showed a great variation among individuals (mean = 4084.28 msec, SD = 2205.7 msec, n = 67). The maximal intensity was reached in the 10th to 15th pulse group, and then maintained until the end of the call. Call duration and number of pulse groups were highly correlated ($r_{Pearson}$ = 0.929, P < 0.0001, 86.4% of explained variance). In regard to coefficient of variation, two categories of acoustic parameters were distinguished (Table 1). Duration and number of pulse groups were very variable; their coefficients were respectively 0.52 and 0.54. In contrast, the ratio "duration/number of pulse groups" and fundamental frequency revealed a low coefficient of variation, respectively 0.22 and 0.09 (Table 1).

Response to familiar and unfamiliar calls

The 22 sampled males did not exhibit differences in their calling activity in comparison with other males in terms of all the studied acoustic parameters (duration t_{Welch} = 0.40, df = 60, P = 0.69; number of pulse groups t_{Welch} = 1.03, df = 62, P = 0.31; fundamental frequency t_{Welch} = 0.25, df = 51, P = 0.81; ratio t_{Welch} = 1.2, df = 62, P = 0.23).

Comparing the male single call with the reply to a familiar call showed no difference in regard to the measured variables: call duration (F = 5.952, P > 0.05),

Table 1.

Principal acoustic parameters for the male Agile frog *Rana dalmatina* observed in western France (n = 67).

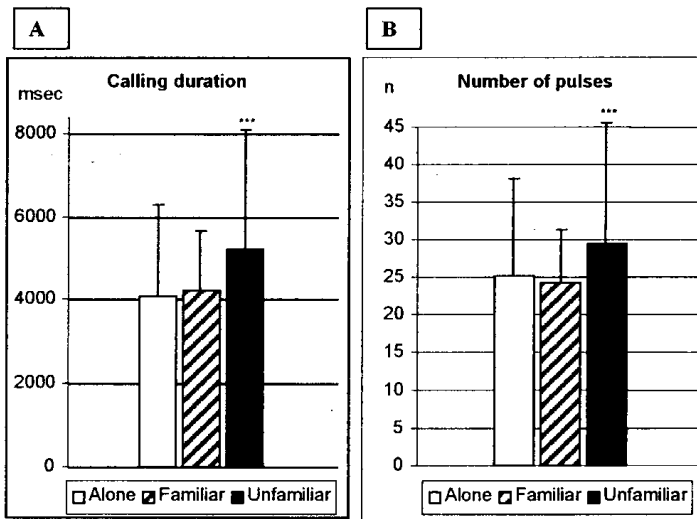|	Duration of sonorous emission (msec)	Number of pulses (np)	Ratio duration/np (msec)	Fundamental frequency (Hz)
Mean	4084	25.19	164.43	682.40
SD	2206	13.14	36.33	61.10
Coefficient of variation	0.54	0.52	0.22	0.09
Range	1416-13793	8-87	71-328	571-865

Fig. 2. — Changes in calling duration (A) and number of pulses (B) when the advertisement call is emitted in response to familiar or unfamiliar acoustic stimuli (n = 24; $P^{***} < 0.01$).

number of pulse groups (F = 5.271, $P > 0.05$), and ratio "duration/number of pulse groups" (F = 3.066, $P > 0.05$). Furthermore, the fundamental frequency remained quasi-identical for each male reply showing that Agile frogs displayed the same advertisement call when the male calls alone or in response to a familiar acoustic stimulus (F = 0.024, $P > 0.05$).

In contrast, in response to unfamiliar advertisement calls, the call duration showed a significant difference for 21 among the 22 individuals, ranging from 2040 msec to 15775 msec (mean = 5204.77 msec, SD = 2891.57 msec; F = 5.952, $P < 0.01$). The number of pulse groups also increased significantly in response to the unfamiliar acoustic stimulus (F = 5.271, $P < 0.05$) (Fig. 2). However, due to the correlation between duration and number of pulse groups, the ratio did not significantly increase in response to the unfamiliar call (F = 3.066, $P > 0.05$). The fundamental frequency did not show any changes (F = 0.024, $P > 0.05$).

DISCUSSION

A large variability is maintained among male Agile frogs in their call charac-
teristics and especially in the call duration and in the number of pulse groups.
Moreover, the Agile frog was able to discriminate between familiar and unfamiliar
advertisement calls when they were presented in natural conditions.

Call variation

Considering the advertisement call as a related fitness trait, Agile frogs are
able to exhibit variable characteristics. Call duration appears as a dynamic parame-
ter that requires a more or less prolonged vocal effort. In fact, call duration may be
influenced by the experience of individuals and that call length constitutes a real
indicator of the male quality (WELCH et al. 1998). This characteristic probably plays
an important role in female choice. Because of the variable energetic involvement
of each male, it could also contribute to the explanation of the high variability of
this parameter. The advertisement call frequency ranged from 571 to 865 Hz. It
remains an individual characteristic that displays few within-male variations (BEE
& GERHARDT 2001b). In hylid frogs, GERHARDT (1994) proposed that dynamic crite-
ria had a coefficient higher than 12%, whereas static criteria had a coefficient
smaller than 4%. With a coefficient of variation reaching 9%, fundamental frequen-
cy was considered to be quasi-static as in numerous anurans (GERHARDT 1991,
1994; BOSCH & MARQUEZ 1996). The call generally depended upon animal size with
the oldest and most experienced male often displaying the lowest frequency (MAR-
QUEZ & BOSCH 1997a, 1997b) and the temperature (SCHNEIDER et al. 1988). This sta-
tic parameter presented a low coefficient of variation (GERHARDT 1991). In accor-
dance with SCHNEIDER et al. (1988), we observed that the number of pulse groups
was directly related to call duration in such a way that the ratio between the call
duration and the pulse groups number remains approximately constant in *Rana
dalmatina*. The ratio between duration and number of pulse groups characterised
the specific call in the Agile frog and could play a determining role in the specific
mate recognition system (PATERSON 1992).

Call recognition

Call characteristics only differed slightly when a male replied to a neighbour
whereas the call duration and the number of pulses significantly increased in
response to an advertisement from a stranger. Such a difference emphasises that
male Agile frogs are able to discriminate between familiar and unfamiliar acoustic
stimuli. Resident males shifted call repetition and duration to a maximum level
when they replied to an intruder. A similar longer call was also observed by WELLS
(1988) and WAGNER (1989). The expansion of call duration acted as a reinforcement
of intimidation contributing to the resident primacy of site occupation (O'NEILL
1983). By increasing call duration, the male seemed to enhance his territorial
defence. On the other hand, frogs may become accustomed to the conspecific activ-
ity by stimuli repetition (BEE & GERHARDT 2001a) and studies on *Rana catesbiana*
(DAVIS 1987) and *Rana clamitans* (OWEN & PERRILL 1998), showed a decremental
response when frogs were faced with familiar individuals. This habituation has

been supposed to play a role in "the dear enemy" effect (BROOKS & FALLS 1975). It results in low aggressiveness towards neighbours compared with strangers. The "dear enemy" phenomenon is explained by the relatively low threat presented by neighbours while strangers represent a high risk of loosing the breeding place (TEMELES 1994). According to the payoff asymmetry hypothesis (KREBS 1982), it can be predicted that a male resident has better reproductive success than a new arrival or a male recently territorialized. In this context of discrimination, *Rana dalmatina* exhibits a reserve of plasticity in its advertisement call. In this territorial species, for which defence behaviour is generally strict, this plasticity is expressed when males are confronted by unfamiliar conspecifics. Indeed plasticity allowed males to maximise the time spent in producing advertisement calls and to attract female by adjusting their behaviour to involve changes in male spacing within choruses.

Call duration and repetition entails a significant energetic expenditure (RYAN 1988, SCHWARTZ et al. 1995), an increased predation risk (LODÉ 1996) and it appears to be an indicator of reproductive investment according to the investment competition model (WELLS & TAIGEN 1986, RYAN 1988). In treefrogs, *Hyla regilla*, females prefer long advertisement calls (BRENOWITZ & ROSE 1999). By reducing the calling cost in the presence of a familiar, *Rana dalmatina* avoids expensive energetic expenditure, as in *Crinia georgiana*, in which males tended also to produce calls that attracted females as much as other males did, without wasting energy (GERHARDT et al. 2000).

CONCLUSIONS

Our results suggest that neighbour recognition may influence intraspecific tolerance and reduce competitive interactions between males. This effect was also amplified by the breeding site tenacity exhibited by most anurans (READING et al. 1991). The maintenance of such a variability in call structure and change in call characteristics as a response to unfamiliar conspecific may result from both strong competitive interactions and territorial breeding behaviour while familiarity could favour tolerance in Agile frogs. The discrimination between familiar and unfamiliar advertisement calls may be considered as a cognitive process, involving a trade off between territoriality and tolerance. Then, reduced aggression between territorial neighbours could be partially mediated by habituation to a neighbour's signals (BEE & GERHARDT 2001c).

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