Fluctuating asymmetries and advertisement call variation in the cricket frog, *Acris crepitans*

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We used an anuran acoustic communication system to test a prediction of the "fluctuating asymmetries/good genes" hypothesis that females prefer more symmetric mates because symmetry indicates genetic quality. Mate preferences of female cricket frogs (*Acris crepitans*) can be influenced by three call characters: dominant frequency, numbers of pulses per call, and number of pulse groups per call. We tested the hypothesis that these preferences result in females preferring more symmetric males. We measured fluctuating asymmetries of characters not involved with the communication system (head and tibia), and those involved in signal production (laryngeal characters) and signal reception (aural characters). We determined whether the asymmetries in these characters were related to the three variables that enhance call attractiveness. Most of the multiple regression models showed no significant association between the fluctuating asymmetries of characters and any of the calls. The regression of head and tibia fluctuating asymmetry on pulse number was significant, but partial regression coefficients revealed that more pulses were associated with a more symmetric head length and a less symmetric tibia length. Our findings provide little or no support for the fluctuating asymmetries/good genes hypothesis. We emphasize, however, that this hypothesis should not be abandoned based on negative results of a single study, but deserves further scrutiny. *Key words: Acris crepitans,* anurans, calls, communication, cricket frogs, fluctuating asymmetries, reproductive behavior, sexual selection. *[Behav Ecol 6:124–131 (1995)]*

Fluctuating asymmetries (FAs) are random variations between sides of otherwise bilaterally symmetrical traits. This form of variation is thought to result from perturbations during development (e.g., Soulé, 1982; Soulé and Cuzin-Roudy, 1982). FAs have been of interest to population geneticists and conservation biologists because the magnitude of an FA is influenced by certain gene–environment interactions. For example, both high levels of homozygosity due to inbreeding and environmental disturbance from pollution are thought to increase levels of FAs (Leary and Allendorf, 1989; Leary et al., 1985; Palmer and Strobeck, 1986; Soulé, 1982).

FAs have recently become a focus for investigating nonrandom patterns of female mating preferences (Liggett et al., 1993; Manning and Hartley, 1991; Møller, 1991, 1992a; Møller and Höglund, 1991; Swaddle and Cuthill, 1994; Thornhill, 1992; Thornhill and Sauer, 1992). One hypothesis, known as "good genes," suggests that mating preferences have evolved because of the advantage accrued to the offspring of females mating with males of superior genetic quality, in which genetic quality usually refers to some component of fitness related to natural selection, such as survival ability (Kirkpatrick and Ryan, 1991; Pomiankowski, 1988; Zahavi, 1975, 1977, 1991). It has been suggested that FAs indicate male genetic quality and that females should prefer males with smaller FAs either by directly evaluating the FA as a signal (Møller, 1992a) or by evaluating signals that are correlated with FAs (Thornhill, 1992). In the latter case, there is no a priori prediction which FAs, if any, will be more informative of male's genetic quality. In this study we use cricket frogs (*Acris crepitans*) to test a prediction of the FA/good genes hypothesis by determining the relationship among FAs, call characters, and female call preferences.

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The system

We have previously examined the function and evolution of advertisement calls in a species of cricket frog, *Acris crepitans* (summarized in Wilczynski and Ryan, in press). As with most frogs, the advertisement call is the primary signal used by males to attract mates, and females rely on these signals to choose both among males of different species and among conspecifics (Rand, 1988). The advertisement call of *A. crepitans* is a click of relatively short duration (approximately 25–125 ms). Each cell contains 4–16 pulses, and these pulses are, in turn, organized into 1–3 pulse groups. The calls are repeated in rapid succession from 25–90 times in a single call group (Figure 1). From the beginning to the end of a call group there is an increase in call duration, number of pulses, and number of the groups of pulses within a call. Males tend to increase all of these parameters in response to calls of other males. Thus Wagner (1991) has suggested the call group grades in function from female attraction to male aggression. The call's dominant frequency, which can range from 2.7–4.0 kHz, does not change predictably over a call group. Males actively vary some call parameters during vocal interactions with other males (Wagner, 1989a,b,c, 1991); thus variation in social situations can obscure variation among individuals.

There is substantial variation in call parameters between the two subspecies of *A. crepitans,* among populations within each subspecies, and among individuals within populations (Ryan and Wilczynski, 1988, 1991; Ryan et al., 1990). There is also variation among populations in the tuning of the auditory nerve (Keddy-Hector et al., 1992; Ryan and Wilczynski, 1988; Wilczynski et al., 1992). The interaction between variation in calls and auditory neurophysiology predicts nonrandom patterns of female call-frequency preference both among and within populations (Ryan and Wilczynski, 1988; Ryan et al., 1992).

Female phonotaxis experiments have revealed three call variables that can influence the attractiveness of the call. In phonotaxis experiments in which a single call parameter is varied while all others are held constant, it was shown that

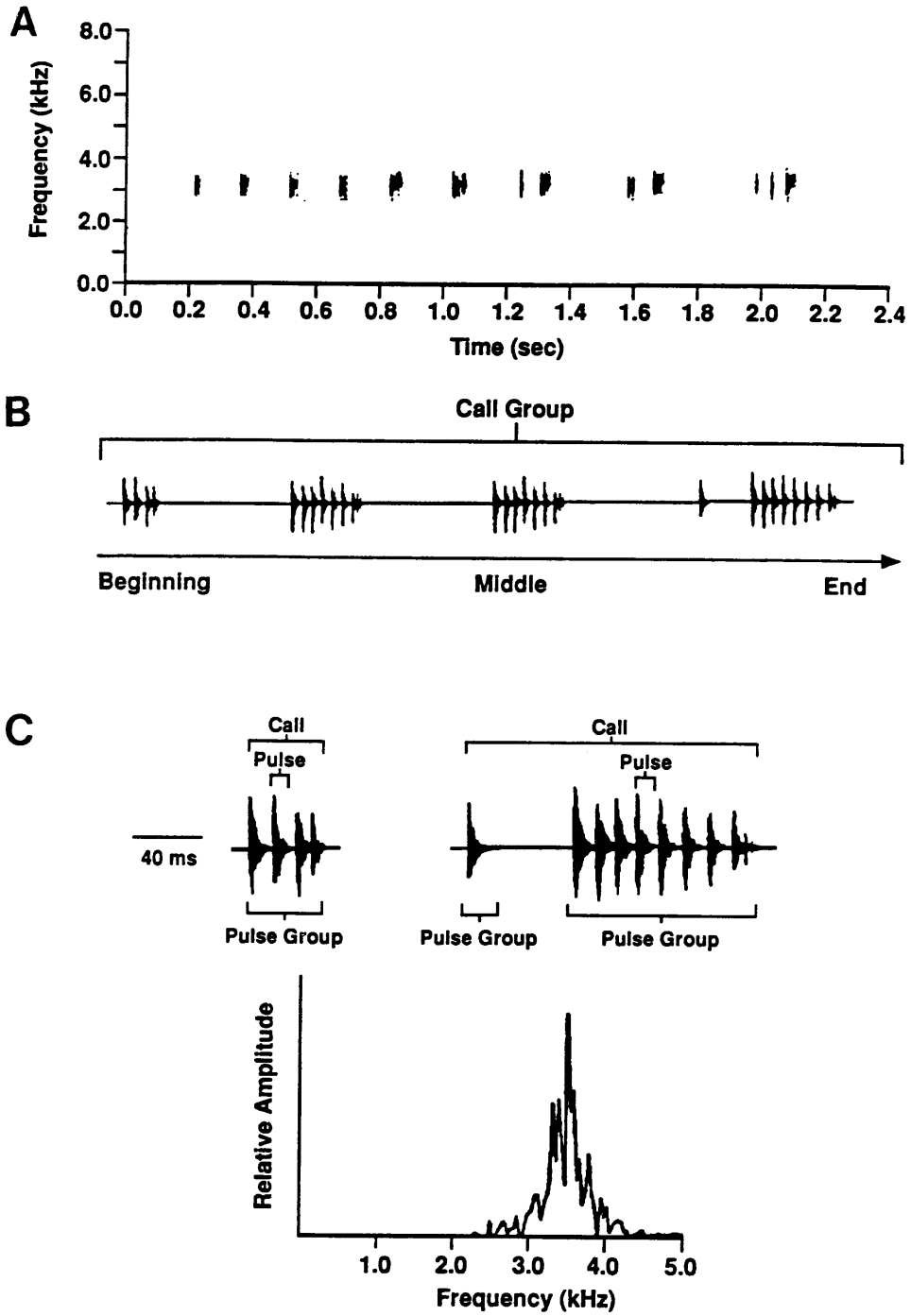

Figure 1
The advertisement call of *Acris crepitans*. (A) Sonogram (frequency versus time) of an entire call group. (B) Oscillogram (amplitude versus time) showing how calls progressively change within a call group, and the pulse and pulse group structure of representative calls from the beginning and end of a call group. (C) Fourier transform (amplitude versus frequency) showing the distribution of energy across frequencies for a single call (modified from Wagner, 1989c).

females are attracted preferentially to calls with dominant frequencies lower than the population mean (Ryan et al., 1992) and to calls with more pulses and more groups of pulses (Wagner, 1991). The interaction effects of these call parameters on preference have not been determined.

The FA/good genes hypothesis predicts that females prefer more symmetric males. Therefore, we determined whether these known female call preferences in cricket frogs were correlated with FAs of a variety of characters and might thus result in females mating with more symmetric males in nature. Most studies of FAs measure only one or a few characters (e.g., Liggett et al., 1993; Thornhill and Sauer, 1992). We measured FAs in 11 morphological characters and determined whether these FAs were correlated with any of the three call characters that influence mating preferences. We feel this larger number of characters would add more credibility to any negative results. These characters fall into three categories: (1) characters not functionally related to the communication system (head length and tibia length); (2) characters related to the signaling system (five laryngeal characters); and (3) characters related to the receiver system (four aural characters). Although we know what set of characters are involved directly in producing and receiving calls, there is no information to suggest how asymmetries in these characters would influence call production or reception. But again, to increase the credibility of any negative results we thought it prudent to assess FAs of characters that varied in the potential functional relationship to the signals being examined.

Table 1
Multiple regression analyses of asymmetries regressed against call characters

Independent variables	Standardized partial regression coefficients (*P*) DomHz[a]	Pulse number	Groups of pulses
Head	0.18 (0.11)	−0.24 (0.02)	−0.10 (0.37)
Tibia	0.03 (0.78)	0.20 (0.06)	0.02 (0.86)
Model	$r = .18$ (.27)	$r = .33$ (.01)	$r = .10$ (.65)
Arytenoid	−0.05 (0.74)	−0.04 (0.83)	0.11 (0.49)
Constrictor	−0.09 (0.63)	−0.02 (0.90)	0.30 (0.11)
Dilator	−0.14 (0.45)	0.11 (0.55)	−0.05 (0.75)
Basal cartilage	0.07 (0.67)	0.10 (0.56)	−0.10 (0.55)
Vocal cords	0.14 (0.44)	0.13 (0.45)	−0.08 (0.66)
Model	$r = .25$ (.82)	$r = .22$ (.88)	$r = .36$ (.41)
Inner ear	−0.32 (0.05)	0.01 (0.95)	0.30 (0.06)
Middle ear	−0.15 (0.36)	−0.35 (0.04)	−0.09 (0.60)
Tympanum	0.12 (0.46)	0.03 (0.85)	−0.09 (0.58)
Columella	−0.04 (0.81)	0.24 (0.15)	0.15 (0.35)
Model	$r = .35$ (.30)	$r = .36$ (.26)	$r = .34$ (.31)

[a] Dominant frequency, adjusted for body size.

The results of the multiple regression model are also shown, in which r is the multiple regression coefficient and P is from an analysis of variance of the model.

METHODS

Measures of characters

We measured three call characters that are known to influence call attraction: number of pulses; groups of pulses; and dominant frequency (Figure 1; Tables 1 and 2); these same calls were analyzed in a different context by Ryan and Wilczynski (1991). Because temporal parameters of calls vary across the call group, we measured these parameters from calls in the beginning, middle, and end of a call group. We again note that features of the call can be influenced by social conditions that we were not able to standardize in the field. Socially induced variation could obscure our results. The dominant frequency was measured for only one call in each call group because this parameter does not change predictably across the call group. For each male, ten calls were measured and the means for each parameter were determined. Only the means are used in the analyses. Calls were analyzed with a DATA 6000 digital waveform analyzer at a sampling rate of 20 kHz (Nyquist frequency = 10 kHz) and with a Uniscan sonograph. Some call parameters vary with temperature; these variables were adjusted to a common temperature using the regression equations in Wagner (1989c).

We measured tibia length and head length of males for whom calls were available; these males had been fixed in 10% formaldehyde and preserved in 70% ethanol. Tibia length was measured with dial calipers from the knee to ankle joint; thus some cartilage is included in the measurement. Head length was measured with calipers from the angle of the jaw to the tip of the snout. Each character for each animal was measured three times. The reliability of repeated measures of the same character was estimated from the intraclass reliability coefficients of a repeated measures analysis of variance. The means of the three repeated measures were used in the analysis. The FA for each character was calculated as $\sqrt{D^2}$, where D is the difference between the left and right side of the character. We used a t test to determine directional asymmetry, and a Pearson's product-moment matrix to determine the extent to which FAs of different characters were correlated.

Measures of characters associated with the larynx, and the external, middle, and inner ear are from McClelland BE, Wilczynski W, and Ryan MJ, unpublished (Figure 2). The head of each animal was isolated by dissection rostral from the laryngeal region and caudal from the eyes. The heads were first decalcified and then dehydrated with ethyl alcohol and xylene

Table 2
Multiple regression analyses of asymmetries regressed against call characters from different parts of the call group

Independent variables	Standardized partial regression coefficients (*P*) Pulse number[a] Beginning	End	Group of pulses[b] Beginning	End
Head	−0.14 (0.20)	−0.19 (0.08)	−0.19 (0.09)	−0.07 (0.53)
Tibia	0.17 (0.12)	0.01 (0.95)	0.03 (0.79)	−0.02 (0.84)
Model	$r = .23$ (.11)	$r = .20$ (.20)	$r = .19$ (.21)	$r = .07$ (.81)
Arytenoid		−0.18 (0.30)	0.11 (0.52)	0.08 (0.62)
Constrictor		−0.07 (0.72)	0.28 (0.12)	0.15 (0.42)
Dilator		0.17 (0.35)	0.02 (0.92)	0.11 (0.52)
Basal cartilage		0.89 (0.70)	−0.04 (0.83)	−0.09 (0.60)
Vocal cords		−0.02 (0.91)	−0.14 (0.41)	−0.80 (0.42)
Model		$r = .24$ (.83)	$r = .36$ (.42)	$r = .29$ (.68)
Inner ear	−0.07 (0.67)	0.18 (0.27)	0.41 (<.01)	0.24 (0.13)
Middle ear	−0.31 (0.06)	−0.14 (0.41)	−0.02 (0.91)	−0.06 (0.71)
Tympanum diameter	0.15 (0.33)	−0.13 (0.42)	−0.14 (0.35)	−0.27 (0.08)
Columella	0.01 (0.99)	0.18 (0.28)	−0.13 (0.39)	0.10 (0.52)
Model	$r = .34$ (.34)	$r = .30$ (.45)	$r = .47$ (.05)	$r = .38$ (.21)

[a] Number of pulses from the beginning and end of the call.

[b] Groups of pulses from the beginning and end of the call.

The results of the multiple regression model are also shown, in which r is the multiple regression coefficient and P is from an analysis of variance of the model. There was no variance in the number of pulses in calls from the beginning of the call group call for those calls associated with laryngeal characters.

before being imbedded in paraffin. Some tissue shrinkage occurs with this procedure, but it should affect both sides of the structure equally. The heads were sectioned in the coronal plane at a width of 25 μm and stained with Pollak's trichome and cresyl violet. The area of every tenth section was measured by projecting the image onto a digitizing pad. Larynx structures were measured in 14–16 sections per animal, and aural structures were measured in 9–10 sections per animal. As these were coronal sections, the left and right sides of each measured structure appeared in each section. Volumes were calculated separately for the two sides of each structure in each animal by treating the area of adjacent measured sections as the bases, and the inter-section distance as the depth, of a cone frustrum, then adding together the calculated volumes of these segments to obtain the total volume of the measured larynx or ear structure. Volumes of the arytenoid cartilages, vocal cords, basal cartilage of the arytenoid, laryngeal constrictor and laryngeal dilator muscles, extra columella, and the middle and inner ear were calculated as was the diameter of the tympanic membrane. FAs were calculated as above. Most of the measurement error in this procedure is likely to be associated with sectioning. Because sections could not be repeated on the same character the measurement error could not be determined; it is possible that such measurement errors could obscure our results. However, because volumes were calculated from a series of sections throughout each measured structure, the impact of small, random errors in the sections should be minimal.

Measurements of all morphological and call characters were conducted in ignorance of corresponding variation for that individual frog. Not all individuals were measured for all characters; specifically, those frogs were sectioned for use in measures of aural and larynx characters were one sample and frogs measured for head and tibia were another sample.

Analysis

We analyzed the character FAs in the three sets because they were involved in the communication system to different degrees. For each set of characters we used a multiple regression analysis to determined the relationship between the FAs of the morphological characters (independent variables) and one of the call variables (dependent variable) that is known to influence female phonotaxis. This yielded a total of nine analyses in one set of results (Table 1) and 12 analyses in another (Table 2). To control experiment-wide error we used the Fisher's combined probability test (Sokal and Rohlf, 1981) to determine the significance of the combined analyses in each set of results (Tables 1 and 2). This generates a statistic which is tested against a chi square distribution with $2N$ degrees of freedom, where N is the number of analyses in each set of results. We felt this approach was preferable to a multivariate multiple regression because those results are often difficult to interpret clearly.

A potential criticism of our analysis in that because calls might change function from the beginning to the end of the call group, combining these functions might obscure relationships between calls, FAs, and preferences. Thus we repeated the above analysis for the number of pulses and the number of pulse groups from calls in the beginning and end of the call group.

RESULTS

The means and standard errors of measures for both sides of each morphological character and the absolute difference between both sides (FA) are shown in Table 3. The FAs ranged from 2% (extra columella volume) to 18% (volume of middle

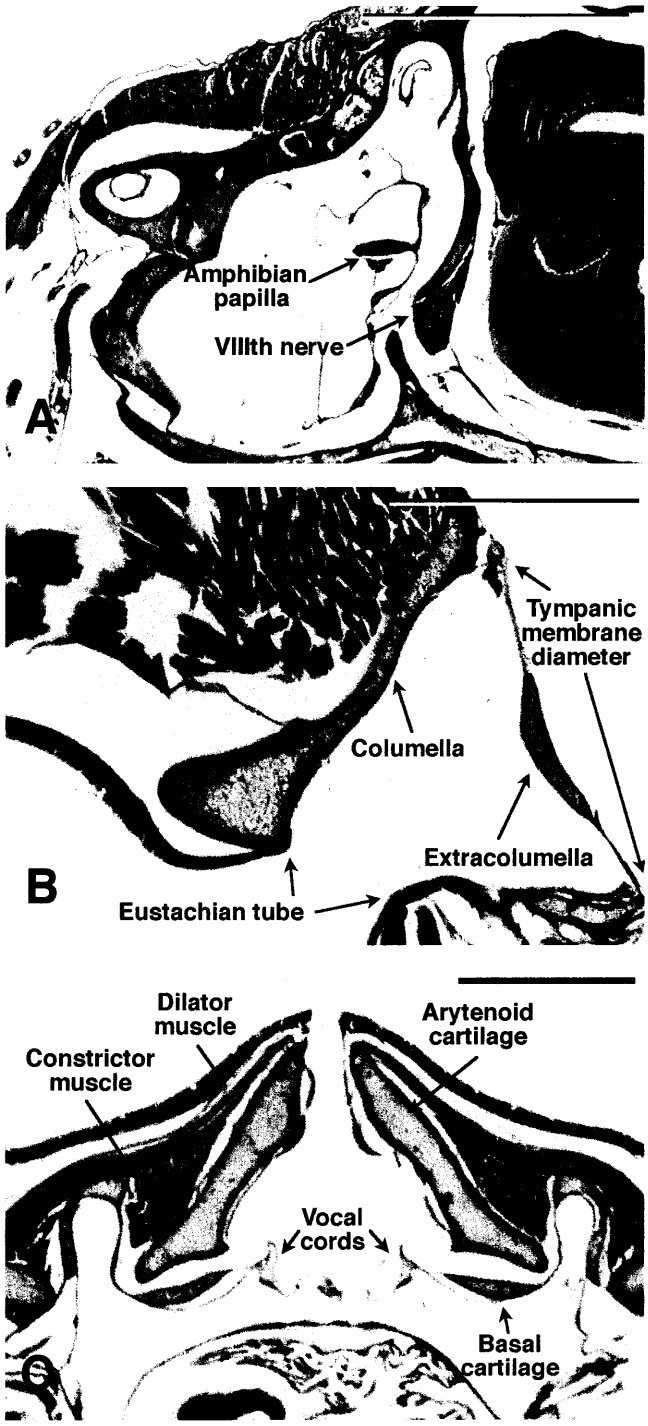

Figure 2
Cross-sections (25 μmm thick) through the (A) inner ear, (B) middle ear, and (C) medial larynx of a male cricket frog (*Acris crepitans*). (A) The inner ear houses components of the peripheral auditory system including the amphibian papilla, basilar papilla (not shown), and VIIIth cranial nerve that transduces and transmits vocal signals. (B) The middle ear cavity is located behind the tympanic membrane, which has an extracolumelar cartilage in its center. Large eustachian tubes connect the middle ear and mouth cavity. (C) Sound is produced by the larynx when air is blown across the vocal cords through the glottal slit setting up a fundamental frequency. Arytenoid cartilages move either passively or actively (via the laryngeal muscles), creating the temporal pulse structure. Basal cartilages of the arytenoid are characteristics of male hylids but have an unknown function. (Scale bar = 1.0 mm.)

Table 3
Summary of character sizes and asymmetries and descriptive statistics

Character	N	FA $\bar{X}$ (SE)	Left $\bar{X}$ (SE)	Right $\bar{X}$ (SE)
Arytenoid cartilage (mm^3)	51	0.033 (0.004)	0.710 (0.021)	0.692 (0.020)
Constrictor muscle (mm^3)	50	0.041 (0.006)	0.541 (0.021)	0.535 (0.020)
Dilator muscle (mm^3)	51	0.022 (0.003)	0.206 (0.009)	0.193 (0.009)
Basal cartilage–arytenoid (mm^3)	51	0.005 (0.001)	0.027 (0.001)	0.029 (0.001)
Inner ear (mm^3)	55	0.081 (0.010)	1.211 (0.033)	1.198 (0.035)
Middle ear (mm^3)	53	0.050 (0.006)	0.277 (0.014)	0.304 (0.015)
Tympanum (mm)	42	0.068 (0.008)	1.039 (0.013)	1.017 (0.012)
Vocal cords (mm^3)	42	0.003 (0.001)	0.052 (0.002)	0.050 (0.002)
Extra columella (mm^3)	55	0.005 (0.001)	0.020 (0.001)	0.021 (0.001)
Head (mm)	85	0.151 (0.014)	7.440 (0.160)	7.393 (0.158)
Tibia (mm)	85	0.104 (0.011)	12.858 (0.277)	12.847 (0.277)

The sample size (N), mean ($\bar{X}$), and standard error (SE) for the absolute difference between the left and right sides (FA) of each character and the left and right side of each character.

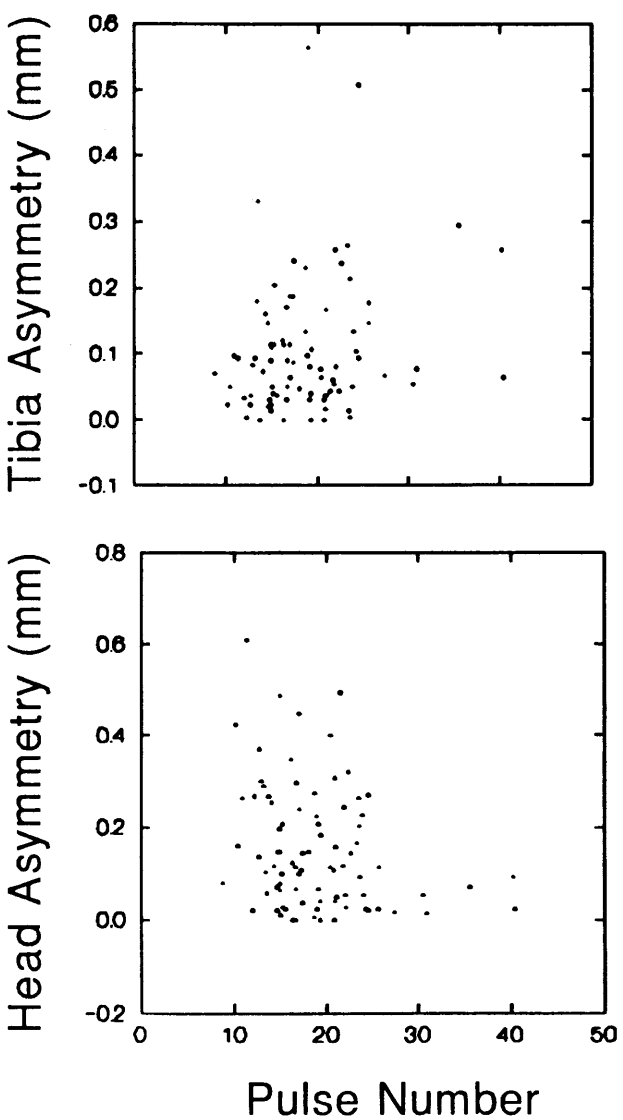

Figure 3
The relationships between the asymmetry in the tibia and head and the number of pulses in the call in *Acris crepitans*.

ear cavity) of the total measures. The repeatability of head and tibia measures was high (intraclass reliability coefficients: left head length, 0.951; right head length, 0.925; left tibia length, 0.993; right tibia length, 0.987; all $p < .001$). FAs were not correlated within individuals. The correlation between FAs of head and tibia was not statistically significant and is negative in sign ($r = -.10$, $p > .10$). Of the 36 possible correlations between characters of the ear and larynx none were statistically significant (r ranges from $-.267$ to .340, all $p > .05$).

There were significant differences between the left and right side in 6 of the 11 characters measured. These included: one of the external characters (head, $t = 2.23$, $p = .028$); four of the five laryngeal characters (the constrictor muscle did not exhibit laterality; $t = -19.32$–3.27, $p = .001$–$.003$), and one of the aural characters, the middle ear volume ($t = -2.59$, $p = .052$). A Fisher's combined probability test suggests that difference between left and right side does not result from experiment-wide error alone ($\chi^2_9 = 77.06$, $p < .05$).

The multiple regression analyses show that when combined the three sets of morphological characters measured do not explain a significant amount of the variation in the three call variables that are known to influence female call preferences (Fisher's combined probability test, $\chi^2_{18} = 22.56$, $p > .10$; Table 1). Of the nine analyses, only the model regressing head and tibia on pulse number was statistically significant when considered in isolation ($p = .01$). Even so, the partial regression coefficients for head and tibia FAs are opposite in sign. Thus, to the degree that female mate choice is influenced by pulse number, females would be choosing males that are more symmetric in head length but less symmetric in tibia length (Figure 3).

None of the other regression models were statistically significant, although there were three partial regression coefficients that were significant or nearly so: the FA of inner ear volume regressed on dominant frequency and groups of pulses, and the FA of middle ear volume regressed on pulse number.

When variation in pulse number and in number of groups of pulses were partitioned within the call group the results were similar (Table 1). The combined results did not show a statistically significant association between call variation and FAs (Fisher's combined probability test, $X^2_{22} = 27.00$, $p > .10$; Table 1). Even when considering the regression models in isolation, and thus increasing the chance of the experiment-

wide error, only one of 12 regression models, inner ear characters on groups of pulses in the beginning of the call, was statistically significant ($p = .05$). In this model only the partial regression of the FA in inner ear volume was significant ($p < .01$), and it is in the direction predicted by the FA/good genes model; males that were more symmetric produce calls more attractive to females. Inner ear volume FA showed the same effect when number of groups of pulses were analyzed for the entire call.

DISCUSSION

As with many other studies of FAs, we show that there are substantial departures from bilateral symmetry in most characters we measured (e.g., Leary and Allendorf, 1989; Leary et al., 1985; Soulé, 1982; Soulé and Cuzin-Roudy, 1982). About half of the characters also showed some consistency in their departure from symmetry. Although some asymmetries were not statistically random in their fluctuations in the samples we measured, we tentatively consider traits with both significant and nonsignificant directionality together because all of these traits appear to be basically bilaterally symmetric, as opposed to directionally asymmetric traits, such as claw size differences in fiddler crabs. We return to this issue of directional asymmetry below.

Despite the existence of substantial asymmetry, our results do not provide strong support for the proposed role of FAs in promoting the evolution of female preferences for genetically superior males (Liggett et al., 1993; Manning and Hartley, 1991; Møller, 1991, 1992a; Møller and Höglund, 1991; Thornhill, 1992; Thornhill and Sauer, 1992).

According to the FA/good genes hypothesis, females might choose genetically superior males either by evaluating the FA in the signal itself, or by preferring display characters that are correlated with FAs. We are testing the predictions of the latter hypothesis. Our previous studies have identified more attractive display characters, and here we determined if preferences for such characters would result in preference for more symmetric males. This approach is similar to that taken by Thornhill and Sauer (1992) and Liggett et al. (1993)—more successful males are first identified and morphological characters are then measured. Our study differs from those, however, in that we rely on our previous studies of phonotactic preferences in this species to assess attractiveness, while in the other studies FAs were compared among males after an episode of mating.

Three call characters are known to influence female mating preferences: dominant frequency, number of pulses, and number of pulse groups (Ryan et al., 1992; Wagner, 1991). The nine multiple regression models analyzed together revealed that variation in FAs of neither head nor tibia, laryngeal characters, nor inner ear characters explained a significant amount of the variation in these three call characters. Even in the model that showed a strong trend toward being significant when considered separately, FAs in head and tibia length regressed on pulse number (Table 1), the partial regression coefficients were in opposite directions; thus one result supported and one result contradicted the FA/good genes hypothesis (Figure 3).

Male cricket frogs increase both the number of pulses and the number of pulse groups in the call in response to calls of other males (Wagner, 1989a,b,c). Within a call group, calls also increase in these parameters from the beginning to the end of the call group. Thus Wagner (1989b) suggested that calls grade in function from the beginning to the end of the call group from primarily female attraction to primarily male aggression. Therefore, we analyzed calls from only the beginning and from only the end of the call group to reduce the possibility of combining in the analyses calls that might be serving different functions. This series of analyses also yielded no support for the FA/good genes hypothesis (Table 1).

Our results suggest that the preferences for call characters exhibited by female cricket frogs do not result in them mating with more symmetric males. Therefore, we cannot support this proposed influence of good genes on the evolution of mating preference in this system. There are two possible sources of error in our study that must be considered. First, measurement error could confound some effects. This might be especially true for volume measures because the error of single dimensions over many sections might be substantial due to variation in the plane of section and other methodological limitations. Repeated measures of head and tibia asymmetry, however, revealed that the error measurement did not represent a significant component of the total variation in FAs among individuals. There might also be a source of measurement error in the calls resulting from variation in social conditions under which the calls were recorded. Second, it is also possible that there are other call factors that influence female preferences and are correlated with FAs. At this point, however, we must reject the hypothesis that choice for good genes mediated by preferences for more symmetric males has influenced the evolution of female acoustic preferences in cricket frogs.

Although our data do not conform to the predictions of the FA/good genes hypothesis, several other studies have supported this hypothesis. While the FA/good genes hypothesis might yield new insights into the evolution of female mate choice, we suggest two cautionary notes.

Most of the studies supporting the FA/good genes hypothesis examine symmetries in the signal itself (Manning and Hartley, 1991; Møller, 1991, 1992a; Møller and Höglund, 1991). Although preference for more symmetric signals is consistent with the FA/good genes hypothesis we suggest an alternative hypothesis that should be investigated. It is possible that some sensory mechanisms might make symmetric signals easier to perceive (Pomerantz and Kubovy, 1986). This in itself would not reject the FA/good genes hypothesis; one could argue that these sensory mechanisms or constraints evolved because of the genetic advantages associated with preferring symmetric males. On the other hand, if such sensory constraints were involved in other perceptual tasks besides mate choice, such as in insect–plant interactions, foraging, or learning, this might reject the hypothesis that female mating preferences for symmetric signals evolved by a good genes mechanism. Understanding the sensory mechanisms underlying female mating preferences has provided some important insights into the evolution of mating preferences in other systems (Enquist and Arak, 1993; Ryan, 1990; Swaddle and Cuthill, 1994); investigations of how symmetry affects signal perception might also be a worthwhile pursuit.

In other studies that support the FA/good genes hypothesis, female choice is based on a signal that does not have an obvious dimension of symmetry–asymmetry, but signal quality is correlated with symmetry in another trait. Thornhill (1992) showed that in scorpionflies, females prefer pheromones from males that are more symmetric in wing length. However, wing length is but a single trait and it is difficult to know if this trait is a good indicator of the overall symmetry of the male. Palmer and Strobeck (1986) indicate that within an individual the FA of one trait is not a good predictor of the FA of another trait, even though the average FA of one trait is a good predictor of the average FA of all traits across the population. Our results support this notion; there were no significant correlations between FAs within individuals, and more often than not the nonsignificant relationships were negative in sign.

It seems important to use multiple traits to estimate an in-

dividual's overall symmetry. For example, in our study FAs in head and tibia exhibited significant (or nearly so) associations with pulse number, but these associations were opposite in sign. Had we measured only one of these traits our conclusions would have depended on which was measured; there appears no a priori reason to suspect that one trait more accurately predicts a male's genetic constitution. Thus when FAs of traits are not correlated within an individual, as in this study, there is immediate concern about applying the FA/good genes hypothesis. We suggest that in future studies of this nature, multiple traits be measured and their relationships be quantified, as has been the case in fisheries biology (e.g., Leary et al., 1985). This criticism also applies to symmetries in the signal itself; it would be worthwhile to know how accurately signal symmetry predicts an individual's overall symmetry. Although there have been other criticisms of this hypothesis (Balmford and Thomas, 1992; Borgia and Wilkinson, 1992; but see Møller, 1992b), they have been particular to the systems under investigation. Our concerns are of a more general nature.

The FA/good genes hypothesis is the most recent in a series of hypotheses suggesting that females evolved mating preferences because of genetic advantages related to natural selection, as opposed to the Darwinian theory that female mate choice is arbitrary (Cronin, 1991). Our study of one species offers no support for this hypothesis. We must remember, however, that among species female preferences can be guided by different criteria that are more or less appropriate in different communication systems. For example, to our knowledge this is the only study in which an acoustic communication system has been used to test the FA/good genes hypothesis (although studies with negative results can be notoriously difficult to publish). It is possible that sounds are inherently more variable than tail size or pheromones, and thus might be less likely to be used by females to assess symmetry. Furthermore, despite the fact that we measured what would classically be considered bilaterally symmetric traits, half of these traits did not have truly fluctuating asymmetries; there was a tendency for one side to be larger than the other, although the larger side was not always constant among traits. Thus it is possible that some of the traits we measured were not appropriate for a test of the FA/good genes hypothesis. Nevertheless, our conclusions are the same whether or not our analyses are restricted to traits with statistically nonsignificant differences between the left and right side. Thus we offer our results as a single case of non-support; we do not argue that the general notion of the FA/good genes hypothesis be rejected based on our single study. We do emphasize, however, that although the FA/good genes hypothesis is an intriguing possibility with some empirical support, as with most good genes hypotheses it might be difficult to eliminate alternative hypotheses (Kirkpatrick and Ryan, 1991).

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