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ORIGINAL ARTICLE

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No evidence for female mate choice based on genetic similarity in the túngara frog *Physalaemus pustulosus*

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Abstract In most sexually reproducing animals, the behavior of one or both sexes during courtship critically influences the success at mating of the opposite sex. This behavior is often interpreted as "mate choice," and there is great interest in why such choices are exercised. The explanation for the evolution of mate choice that has received the most attention and generated the most controversy is based on assumed genetic effects. In this study, we investigated whether female túngara frogs, which choose mates based on acoustic cues, have a preference for genetically less related males. Specifically, we determine if there is disassortive mating based on microsatellite markers, if there is information in the advertisement call that could be used to assess genetic similarity, and if females exhibit acoustic-based mating preferences that would promote choice for genetic diversity. Using seven microsatellite markers, we found no correlation of male call similarity and male genetic relatedness. Female choice experiments showed no female preference for calls of less related males, and there was no evidence for inbreeding avoidance in the field. Our results do not support the hypothesis of mate choice based on information about genetic relatedness conveyed by acoustic signals in túngara frogs.

Keywords Animal communication · Mate choice · Relatedness · Microsatellite marker · Sexual selection

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Introduction

In most sexually reproducing animals, the behavior of one or both sexes during courtship critically influences the success at mating of the opposite sex. This behavior is often interpreted as a "mate choice," and there is great interest in why such choices are exercised (Kirkpatrick and Ryan 1991; Andersson 1994; Ryan 1997; Kokko et al. 2003). The competing, often not mutually exclusive, hypotheses can be classified in several categories. In many cases, females gain direct benefits in fecundity due to the chosen male providing superior paternal care or having access to superior resources (Andersson 1994; Ryan 1997; Kokko et al. 2003). A second explanation for female choice is that current mate-choice patterns are incidental consequences of behavior that evolved in a different context. Female preferences for conspecific vs heterospecific males, for example, can incidentally influence their preferences among conspecific males (Gerhardt 1994; Pfennig 1998; Hankison and Morris 2003). Similarly, sensory biases that exist for reasons outside of the context of conspecific mate choice or mating behavior in general can influence female preferences for novel cues and allow males to evolve traits that exploit these preexisting biases (Ryan 1990, 1998; Endler 1992; Shaw 1995; Endler and Basolo 1998). A third explanation for female choice evolution is the hypothesis of Fisher (1930) of runaway sexual selection. He suggested that male traits and female preferences become genetically correlated, and that female preferences evolve not because they provide any fecundity advantage to the females, but as an incidental effect of their correlation to the male trait (see also Lande 1981; Kirkpatrick 1982).

The explanation for the evolution of female mate choice that has received greatest attention and generated the most controversy is based on assumed heritable survival effects in the offspring. Originating with the handicap principle of Zahavi (1975) and culminating in a series of revisions and emendations (e.g., Grafen 1990; Pomiankowski 1988; Zahavi and Zahavi 1997; Hamilton and Zuk 1982; reviewed in Maynard Smith and Harper 2003), this hypothesis posits that female choice evolved under selection to choose males with superior genotypes for survivorship. This is a difficult hypothesis to support unequivocally because it requires the demonstration of a paternal effect on juvenile survivorship. Nevertheless, some studies have provided strong support when measuring various components of survivorship (Petrie 1994; Raberg et al. 2003; Welch et al. 1998). However, there is one form of female choice based on genetic quality that has been well demonstrated, and that is mate choice that results in species recognition.

It is well known that interspecific mating often results in decreased fecundity or in offspring with decreased viability due to the genomes of the two hybridizing species not being compatible (Dobzhansky 1975). However, mate choice for genetic compatibility is not restricted to contrasts between species. Since the demonstration of Yamazaki et al. (1976) that female mate choice in mice is influenced by variation in the major histocompatibility complex (MHC), a series of studies have extended these inquiries to a diverse number of taxa such as other rodents (Penn and Potts 1999) and humans (Wedekind et al. 1995; Wedekind and Furi 1997) as well as nonmammalian taxa such as fishes (Landry et al. 2001; Milinski 2003) and birds (Zelano and Edwards 2002; reviewed in Bernatchez and Landry 2003). In addition, some other studies have documented mate choice for genetically less similar individuals based on factors other than MHC-related odor cues (e.g., Waldman et al. 1992; Waldman and Tocher 1998) or odor cues related to the *t* complex in rodents (Lenington et al. 1994).

In this study, we ask if female mate preferences in túngara frogs, *Physalaemus pustulosus*, which are based primarily on acoustic cues, result in choice of males that are genetically dissimilar. Specifically, we investigated microsatellite markers to determine if there is disassortative mating, if there is information in the advertisement call that could be used to assess genetic similarity, and if females exhibit acoustic-based mating preferences that could promote choice for genetic complementarity. If this hypothesis can be substantiated for *P. pustulosus*, it would parallel a similar phenomenon described for American toads, *Bufo americanus*, by Waldman et al. (1992) and Waldman and Tocher (1998), and would encourage the study of choice for genetic complementarity in other systems that appear not to be mediated by MHC. If the hypothesis is not supported, it might encourage comparative studies to understand the evolutionary histories that do and do not produce choice for genetic complementarity in sexual communication systems.

Materials and methods

Túngara frogs (*P. pustulosus*) are abundant and widespread in Middle America. They have a lek mating system in which males aggregate in temporary ponds and advertise for mates by calling. The male mating call consists of two components. The initial component is a frequency sweep (whine) that can be produced alone and is necessary and sufficient for species recognition (simple call). The whine can be followed by one or more much shorter components (chucks), and together, the whine and chuck constitute the complex call (Ryan 1985).

Females visit male choruses to select a mate. All recordings of male advertisement calls in this study were made at such choruses, and females used in phonotaxis experiments were also collected at these sites, usually after they had chosen a mate and were in amplexus. All frogs used in this study were captured in a 10-km range around Gamboa, Panama (09°07′ 0”N, 79°41′53”W; Lampert et al. 2003). They were released immediately after being marked individually (toe clips) at the same place where they had been found.

Relatedness estimates

All toe clips were stored in a 20% EDTA/sarcosyl buffer for microsatellite marker analysis in Austin, TX. DNA extractions were performed with a DNeasy extraction tissue kit (Qiagen). Seven highly polymorphic microsatellite loci (CA 120, CA 298, A #3.11, A #19.11, C #30.11, ATG 159, and ATG 263; Pröhl et al. 2002) were used to individually genotype all animals. We used fluorescent polymerase chain reaction (PCR) and an ABI Prism 3100 capillary sequencer (Applied Biosystems) as described in Lampert et al. (2003) to estimate allele sizes. Individual pairwise relatedness was estimated using the program Relatedness 5.0.8 (Goodnight and Queller 1995). This measure estimates pairwise relatedness based on allele frequencies (Queller and Goodnight 1989). Estimated relatedness values vary between −1 and +1. Full siblings are expected to have a mean relatedness of 0.5; half siblings are, on average, 0.25, and unrelated individuals are 0.0 related (Blouin et al. 1995). We used the simulation function in the program Kinship 1.3.1 (Goodnight and Queller 1996) to estimate the expected relatedness of 1,000 full siblings, 1,000 half-siblings, and 1,000 unrelated individuals to evaluate the reliability of our microsatellite loci and to create reference points on the Queller and Goodnight R scale.

Mating call recording and analyses

In July 1996, we recorded 48 males from one population in Gamboa, Panama. Calls were recorded with a Marantz PMD 420 recorder and a Sennheiser ME 80 microphone (details on recording and analysis in Ryan and Rand 2003). At least five complex calls (whine plus one chuck) per male

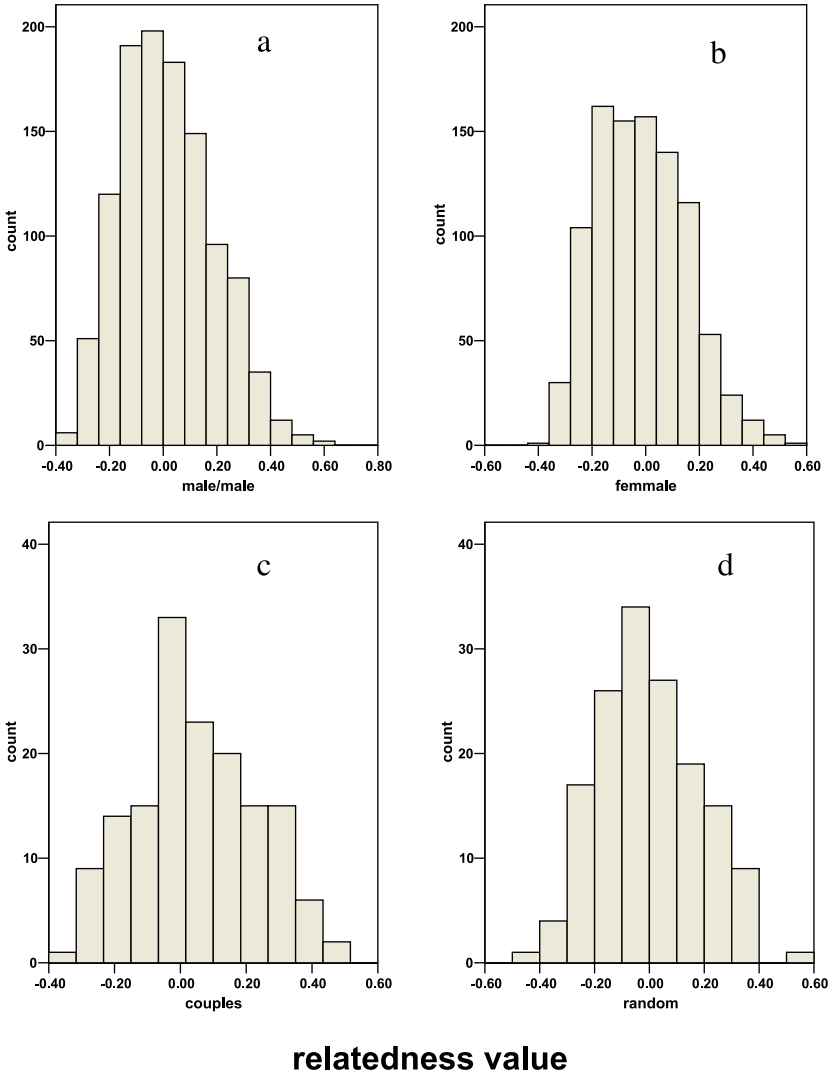

Fig. 1 Histograms of pairwise relatedness values (*x*-axis, relatedness value following Queller and Goodnight 1989) for **a** Gamboa males to each other (*malemale*), **b** females vs males in the female choice tests (*femmales*), **c** couples found in the field (*couples*), **d** randomly generated couples (*random*)

were recorded. The calls were digitized at a sampling rate of 20 kHz and analyzed using the program Signal. Fifteen call parameters were measured (abbreviations): duration of the entire complex call (calldur), dominant frequency of the entire call (callhz), duration of the chuck (ckdur), dominant frequency of the chuck (ckdomhz), duration of the whine (whdur), initial frequency of the fundamental frequency of the whine (inithz), final frequency of the fundamental frequency of the whine (endhz), time to mid-frequency of the fundamental frequency of the whine (hfhz), falltime of the whine (fall), time to half amplitude of the fall from the call's end (hffall), rise time of the whine (rise), time to half amplitude of the rise (hfrise), maximum frequency of fundamental frequency of the call (maxhz), time to maximum frequency of the whine (timmxhz), and peak amplitude of chuck divided by peak amplitude of the whine (relamp). For the analysis of call similarities, we arbitrarily selected one call per male (the third call in the recorded sequence) of all the recorded calls. We transformed call characters into *Z* scores, and we calculated the

Table 1 Descriptive statistics of pairwise relatedness values (Queller and Goodnight 1989) for males vs males in the call–genetic relatedness comparison, females from the female choice tests toward males from the call–genetic relatedness comparison, field couples (couples), randomly generated pairs (random), and females vs. females in the female choice tests

	Male/male	Female/male	Couples	Random	Female/female
Number of cases	1,128	960	153	153	190
Minimum	−0.378	−0.402	−0.346	−0.499	−0.347
Maximum	0.630	0.563	0.460	0.511	0.515
Median	−0.002	−0.023	0.036	−0.021	−0.005
Mean	0.015	−0.015	0.049	0.000	−0.003
Standard deviation	0.172	0.166	0.185	0.183	0.180

Table 2 Mantel test results for the correlation of male call traits and male individual pairwise relatedness

	g	*Z*	*r*	*p*
Overall	0.380	3112.664	0.024	0.36
Calldur	0.0162	2601.006	0.001	0.49
Callhz	−0.357	2636.119	0.050	0.26
Ckdur	−0.102	2475.117	−0.006	0.41
Ckdomhz	1.143	2309.975	0.065	0.17
Whdur	0.526	2621.100	0.282	0.26
Inithz	−0.056	2495.560	−0.003	0.43
Endhz	−0.916	32434.64	−0.029	0.13
Hfhz	0.061	2396.638	0.004	0.45
Fall	−0.412	2614.435	−0.020	0.35
Hffall	***2.28***	***2689.023***	***0.110***	***0.01***
Rise	0.244	2300.576	0.015	0.45
Hfrise	0.645	2005.490	0.045	0.28
Maxhz	−0.834	2579.371	0.045	0.24
Timmxhz	1.202	2318.979	0.053	0.08
Relamp	−1.384	2502.253	−0.081	0.13

p values were derived from 1,000 randomizations. Significant correlations previous to Bonferroni correction are given in bold, italic typeface. After Bonferroni correction, none of the correlations was significant (abbreviations of call parameters are given in the text)

Euclidean distances as a measure of overall call similarity and similarity of each call character among males. As the data matched the assumptions of Mantel tests (e.g., linear correlation between variables, same scales within matrices, and independent observation pairs) call similarities were compared to the genetic similarity with Mantel tests (Mantel Version 2.0; Liedloff 1999). In addition, we used Ritland's Marq program (h^2 only model; Lynch and Ritland (1999) measure of relatedness) to estimate heritability of mating call characteristics in this field population (Ritland and Ritland 1996; Mousseau et al. 1998). To enable a direct comparison between this study and the results of the studies of Waldman et al. (1992), we additionally calculated a simple allele-sharing index (Sxy) between individual males (Sxy = number of shared alleles divided by the average number of alleles scored).

Table 3 Mantel test results for a correlation of genetic relatedness and the hffall in all other recorded calls (1, 2, 4, and 5)

Hffall for all calls				
Call number	*g*	*Z*	*r*	*p*
1	0.843	1,933.43	0.060	0.214
2	1.931	2,634.62	0.108	0.018
3	2.280	2,689.02	0.110	0.010
4	2.199	2,676.34	0.121	0.010
5	0.642	2,662.44	0.029	0.230

No results were significant after Bonferroni correction ($p<0.01$)

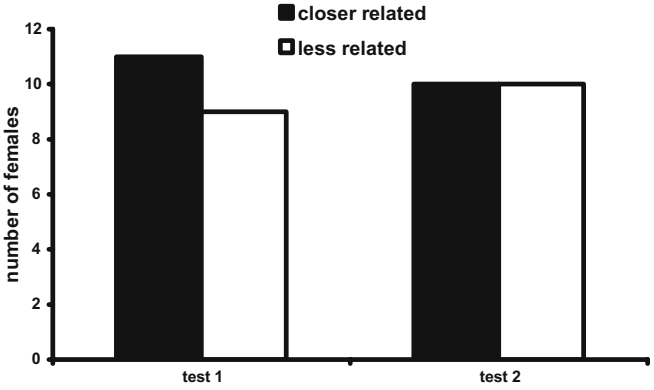

Fig. 2 Results of the female choice tests: *test 1*, random pairs of males; *test 2*, one out of four chosen to be the least related male pairs

Female phonotaxis

Phonotaxis experiments were conducted in an acoustic chamber (1.8×2.7 m) with two small speakers (Cambridge Soundworks) placed in the center of the shorter walls opposite one another along the longer axis of the chamber (distance between the speakers about 2.5 m). The females were released at the center of the chamber. Each call used in the tests consisted of a whine plus one chuck. The calls were chosen from a set of 300 natural calls from this population that were recorded and analyzed for a previous study (Ryan and Rand 2003). Calls were adjusted to a maximum whine amplitude of 82 dB sound pressure level (SPL) (re. 20 μPa) at the release point of the females, which mimics the male's call at a distance of approximately 1.0 m. The chuck amplitude was maintained at the original relative amplitude to the whine. Calls were broadcasted alternately from the two speakers on either side of the test arena. The females were observed remotely using a video camera and infrared light. A choice was considered valid if the female approached a speaker within 10 cm without following the chamber walls (details on the testing rules in Ryan and Rand 2003).

Twenty-five mated females were collected in August 2003 from the same location where we had recorded the males' calls. Each female was presented with a different pair of calls that were randomly chosen from the 48 males used in the previous call-relatedness study (test 1). In addition, each female was presented with one of four male pairs that were found to be rather unrelated to enhance the relatedness bias between the two males and the female (test 2). To control for the females' general mating motivation, the females were presented with a choice between a synthetic whine and whine–chuck before and after the relatedness tests. Only if she made a choice in both whine/whine–chuck tests were the choices that she made in the intervening relatedness tests counted valid. Females were toe-clipped after the behavioral tests to prevent retesting and released within the next 12 h. All frogs were handled and toe-clipped in accordance with the guide for use of live amphibians and reptiles in field research by the American Society of Ichthyologists and Herpetologists (ASIH; http://www.asih.org/pubs/herpcoll.html).

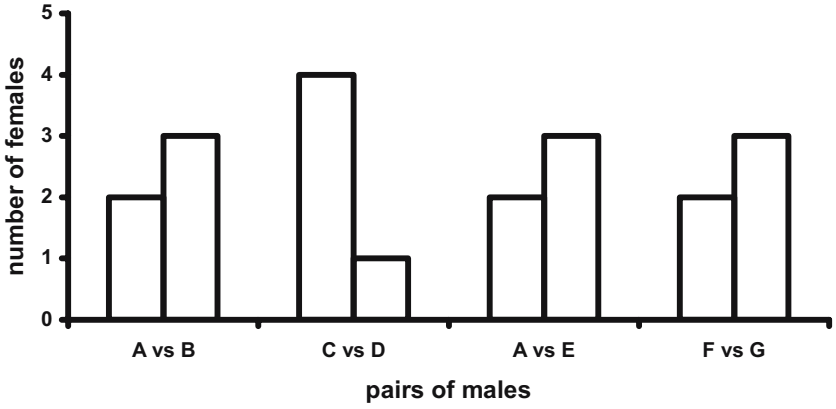

Fig. 3 Choices by females for the calls of individuals males of the four pairs of males used in test 2. *Letters* represent the different males (*A*–*G*). Five females were tested with each pair of males

The females' relatedness toward the males was estimated only after the behavior tests were done.

Relatedness of couples in the field

To estimate the relatedness of mated frogs in the field, we collected amplectant pairs from 15 different breeding sites around Gamboa. All frogs were collected between June and August 2002 between 2000 and 0100 hours. Whenever possible, we collected all other túngara frogs present at the ponds at that time to estimate the full range of relatedness and the number of males available to the females.

Results

Male relatedness and heritability of male call characteristics

Males captured and recorded in 1996 in Gamboa were not closely related (Fig. 1a, Table 1). The average relatedness of males recorded and captured in Gamboa in 1996 was 0.015, qualifying them as "unrelated." The kinship simulations of relatedness of half-siblings, full siblings, and unrelated individuals based on the allele frequencies found in the population confirmed that the males' relatedness was within the range of unrelated individuals [given are mean±standard deviation full sibling, R=0.501±0.174; half-siblings, R=0.251±0.180; and unrelated, R=0.011±0.164].

Correlations between the matrices generated for male genetic relatedness (Relatedness 5.0.8) and male call similarity revealed no significant relationship between individual relatedness and single call parameters (Bonferroni correction for multiple testing) (Tables 2 and 3). The heritability estimates by Ritland and Ritland (1996) showed no significant heritability for any call character (data not shown). The pairwise relatedness between males calculated with an unweighted allele-sharing index, Sxy, varied between 0 and 0.9. Genetic distances obtained with the Sxy measure were highly correlated with the genetic distance obtained with the Queller and Goodnight method (r=0.766, p<0.001). Consequently, none of the tested call parameters was significantly correlated with the genetic relatedness calculated as Sxy (all r values <0.08, all p values >0.07).

Female choice in phonotaxis

The females in the choice tests were not closely related to each other or to the males involved (Table 1, Fig. 1b). The majority of all females chose in all four behavioral tests (80%). The choosing females showed no significant preference for the mating calls of the less related males (test 1:

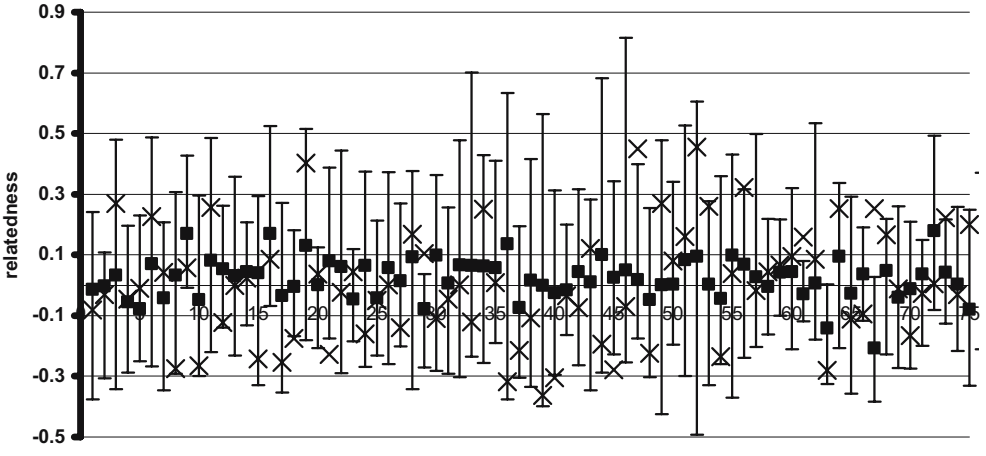

Fig. 4 Mate choices of 76 females (*x*-axis). *Bars* indicate the range of relatedness (*y*-axis) of available partners during that night. *Squares* indicate the mean relatedness of the males towards this particular female. *Crosses* indicate the relatedness of the actually chosen male

X^2=0.100; *df*=1; *p*=0.75; test 2: X^2=0.000; *df*=1; *p*=1.0). In the first experiment (mean male relatedness 0.0147± 0.186), where the females were presented with randomly chosen pairs of male calls, 55% (lower 95% confidence interval limit 31.5%; upper 95% confidence interval limit 76.9%) of the females preferred the more closely related male's call (Fig. 2). In the second experiment, in which females were tested with calls of males (A–G) that were preselected to enhance differences in relatedness (mean male relatedness −0.2365±0.035), 50% (lower 95% confidence interval limit 27.2%; upper 95% confidence interval limit 72.8%) of the females chose the call of the more related males (Fig. 2). In addition, in test 2, there was no single unanimously preferred call in a pair, as would be expected if females had a preference for a specific genotype (Fig. 3). Unfortunately, sample size was too low to test for any statistically significant preference.

Relatedness of couples in the field

A total number of 153 couples were found at the 15 different breeding sites. The average pairwise relatedness of couples varied between −0.346 and +0.460, with a mean value of 0.049±0.185. Randomly generated couples had an average relatedness of 0.000 (Table 1). Túngara frog couples did not show a significantly different distribution of individual relatedness than randomly generated pairs (Kolmogorov Smirnov test, two-sided *p*=0.16; Fig. 1c,d). Out of the 76 matings investigated in more detail (all other males present at the pond during that night were toe-clipped as well) 33 females mated with males that were more closely related to them than the average of all males available (Fig. 4). Statistically, there was no difference between the chosen mates relatedness toward the female and the rest of the males available [chosen partner relatedness 0.034±0.175; rest of males 0.023±0.070; *t* test: *T*=0.511, *df*=150, two-sided *p*=0.610]. A greater number of males available during the night did not seem to alter the females' mating decisions toward less related males (Pearson correlation of number of males available and relatedness of males: *r*=0.02, *N*=76, *p*=0.887). Unmated and mated males did not differ in their level of overall heterozygosity [mean heterozygosity for seven loci (%): unmated Ho=0.722±0.108; mated Ho=0.718± 0.113].

Discussion

Our study finds no evidence that mate choice based on genetic relatedness, as estimated by microsatellite variation, influences mating patterns in the population of túngara frogs we studied. There is no pattern of negative assortative mating by relatedness. It is possible, however, that females have preferences for less related individuals, but that these preferences are compromised in the wild; that is, mating success might not be indicative of mate choice. Contrary to this expectation, we find no evidence for any information in the advertisement call that could be used to assess relatedness; in túngara frogs, as in most frogs, the call is the predominant male advertisement signal. Of course, it is possible that such information exists and is merely not captured by the signal parameters that we analyzed. Contrary to this expectation, females show no patterns of phonotactic preferences between calls of males with different relatedness to themselves. Thus, in three separate data sets, mating success, mating call variation, and phonotaxis preferences, we find no hint that female mate preferences and male mating success are influenced in any way by considerations of genetic relatedness.

Comparison to other studies

The aim of our study was to primarily understand factors that influence the evolution of sexual communication systems. More specifically, we were motivated by a series of studies by Waldman et al. (1992) and Waldman and Tocher (1998) presenting strong evidence that female toads, *B. americanus*, preferentially choose to mate with males that are genetically less related to them. Waldman et al. showed that *B. americanus* exhibited negative assortative mating by mitochondrial haplotype in a series of ponds. Further, they showed a significant positive correlation between similarity in several individual parameters of the mating call and the genetic relatedness of males as estimated by shared bands in DNA fingerprints. Finally, Waldman et al. tested phonotaxis preferences of females for pairs of mating calls and showed very strong preferences for calls of males that were genetically less similar to the test females. The main components of our study were purposefully designed to parallel those of Waldman et al.

It is instructive to consider potential differences between the two studies. Some differences are methodological. Our sample sizes were larger in measures of mating success and call-relatedness correlations. Even with a sample size three times larger than theirs, we could not detect any correlation between male genetic similarity and male call character similarity (Waldman et al. found significant correlations within populations with only 15 or 16 individuals per population.). Our approach to measuring mating success and potential call-related correlations were similar with a few exceptions. Waldman et al. used mitochondrial haplotypes to assess relatedness among mating individuals and shared DNA fingerprint bands as a genetic marker in testing the hypothesis that calls indicated relatedness. In both components of our studies, we used seven highly polymorphic microsatellite loci previously developed for studies of túngara frogs' by Pröhl et al. (2002). Microsatellite analyses utilize a different approach to estimating genetic diversity than mtDNA sequencing. Since microsatellites are biparentally inherited codominant markers, they resolve relatedness at a much finer scale than maternally inherited

mtDNA. Using mtDNA, Waldman et al. were able to show high levels of population differentiation at a small geographical scale (1–2 km). They also showed significantly fewer matings than expected between individuals sharing the same mtDNA haplotype (maternal siblings). Although we found statistically significant levels of genetic differentiation between breeding sites at a scale of 3–4 km and a slight male bias in dispersal in a previous study, Fst values [fixation index (subpopulation compared to total)] still suggested substantial levels of gene flow between populations of túngara frogs (Lampert et al. 2003). In this study, microsatellite estimates did not reveal any evidence for inbreeding avoidance in field populations of túngara frogs. Thus, we are unable to detect genetically biased patterns of mate choice despite larger sample sizes and more sensitive tools than those used by Waldman et al.

While in the observation of mating success, our estimated relatedness values were much finer-scaled than those utilized by Waldman et al., his estimates of relatedness in male call character heritability and female choice tests based on multilocus DNA fingerprints should allow a higher resolution of relatedness than our seven microsatellite loci. Multilocus fingerprints, however, are prone to some error in relatedness tests due to the uncertainty of bands of the same size actually representing alleles at the same locus. To facilitate a direct comparison of the results of Waldman et al. and our microsatellite data, we estimated unweighted allele-sharing distances (Sxy). As expected, the multilocus DNA fingerprints of Waldman et al. resolved pairwise relatedness at a finer scale than the microsatellites. The range of individual relatedness revealed by the microsatellites, however, was larger (0–0.8) than the range of individual relatedness revealed by Waldman et al. (0–0.5). Although our study included more individuals (48 for one site compared to 15 per site) and covered a wider range of individual relatedness than those covered by Waldman et al., we did not find a significant correlation between call similarity and genetic similarity in túngara frogs. This leads to the conclusion that in *B. americanus*, the correlation of call similarity and genetic relatedness must be much stronger than in *P. pustulosus*, and that call trait inheritance in toads might be based on a rather simple genetic mechanism.

Our phonotaxis studies differed in some ways from those of Waldman et al. We randomly selected pairs of calls from the population, and each of a set of the 20 responding females was tested with a single pair of calls. We feel this is a better assessment of how female choice could be influenced by genetic relatedness in nature, but it is not the strongest test to reveal any potential for such an effect. Therefore, we also tested the same set of females with a pair of calls from males that showed a relatively high difference in genetic relatedness. This last experiment parallels the approach taken by Waldman et al. In neither case, however, were female túngara frogs' preferences for calls influenced by genetic relatedness.

It is possible that the different results of these two studies could be due to critical differences in the study species' biology. There are, for example, important differences in mating systems among these species. Toads have a relatively explosive breeding system (Wells 1977), in which the opportunity for female choice should be less than in the longer-breeding túngara frog. Such a difference, however, would predict a greater, not a lesser, opportunity for female choice to assess genetic differences among males. Another possibility is that female preferences are under selection by other forces that constrain their opportunity for genetic-based mate choice. For example, female túngara frogs choose larger males who tend to fertilize relatively more eggs, and females rely, at least in part, on the spectral characteristics of the male's chuck to assess male size (Ryan 1980, 1983, 1985). Such an immediate benefit to mate choice could be contrary to and override benefits from genetic-based mate choice (Kirkpatrick and Barton 1997). Another possibility is that the need to discriminate against heterospecifics compromises the use of cues that might be useful in mate choice within the species, as has been shown in spadefoot toads by Pfennig (1998). We have no strong evidence that would support or reject such a view.

A final set of explanations for the differences between these studies could derive from differences in population structure. Depending on patterns of philopatry and survivorship, the distribution of relatedness among individuals can differ drastically and significantly constrain or promote the opportunity for genetic-based mate choice. The American toad is a rather long-lived species with high levels of philopatry in both sexes (Wells 1977), which should promote a higher probability of close kin encounters. Túngara frogs seem to suffer high mortality rates during all life stages and are likely to only survive one breeding season. They also breed in temporary ponds and are known to be rather flexible in the choice of their breeding habitat (Marsh et al. 2000). In a former study (Lampert et al. 2003), we found high levels of gene flow between sites and a male bias in dispersal. All these factors might make breeding-pond encounters with close relatives rather unlikely. Low probabilities of encounters with close kin could explain why females did not evolve a genetically based mechanism for adult kin recognition. Unfortunately, we are not able to estimate the costs of inbreeding in these frogs, nor is it known in the American toad.

There is one additional possibility that could explain the difference between these species in the use of genetics in mate choice. American toads exhibit sibling preferences in the tadpole stage (e.g., Waldman and Adler 1979). It is possible that bias against closely related individuals in the adult stage is necessary to counter kin preferences that might be adaptive earlier in life but might be maladaptive later in life. Despite a wealth of information on the natural history of túngara frogs, their propensity for sibling preferences has not been explored.

Genetic-based mate choice and olfactory cues

As mentioned previously, preferences for conspecific vs heterospecific mates are a phenomenon thought to result from strong selection on females to avoid mates with

genomes that are not complementary (Dobzhansky 1975; Coyne and Orr 1998; Mays and Hill 2004). When mate choice is restricted to comparisons among conspecifics, however, such evidence for genetic-based mate choice is less common. It seems to be most widespread in mate choice influenced by variation in MHC and associated odor cues. Differences in the antigen-binding site of class I MHC molecules result in distinctive odors (Yamazaki et al. 1991; Penn 2002), but as of now, we have no evidence that variation in vocalizations correlate with variation in MHC (Zelano and Edwards 2002). At this point, it is difficult to posit a mechanism for MHC heterozygosity recognition mediated by mating calls. We cannot, however, exclude the possibility that female túngara frogs attend to male MHC type. Although it might be unlikely that MHC variation is encoded in the male mating calls, females could detect olfactory cues when coming into close proximity of their potential mate (Waldman and Bishop 2004). Female túngara frogs, for example, approach several males closely before choosing a mate (Ryan 1985). It might be possible that túngara frogs choose mates based on MHC compatibility rather than based on overall genetic relatedness (Landry et al. 2001).

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