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OPPORTUNITY FOR SEXUAL SELECTION AND EFFECTIVE POPULATION SIZE IN THE LEK-BREEDING EUROPEAN TREEFROG (*HYLA ARBOREA*).

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Sexual selection in lek-breeding species might drastically lower male effective population size, with potentially important consequences for evolutionary and conservation biology. Using field-monitoring and parental-assignment methods, we analyzed sex-specific variances in breeding success in a population of European treefrogs, to (1) help understanding the dynamics of genetic variance at sex-specific loci, and (2) better quantify the risk posed by genetic drift in this species locally endangered by habitat fragmentation. The variance in male mating success turned out to be markedly lower than values obtained from other amphibian species with polygamous mating systems. The ratio of effective breeding size to census breeding size was only slightly lower in males (0.44) than in females (0.57), in line with the patterns of genetic diversity previously reported from *H. arborea* sex chromosomes. Combining our results with data on age at maturity and adult survival, we show that the negative effect of the mating system is furthermore compensated by the effect of delayed maturity, so that the estimated instantaneous effective size broadly corresponded to census breeding size. We conclude that the lek-breeding system of treefrogs impacts only weakly the patterns of genetic diversity on sex-linked genes and the ability of natural populations to resist genetic drift.

KEY WORDS: Amphibians, effective breeding size, genetic drift, *Hyla*, lek, opportunity for selection, sex chromosomes.

The outcome of all major evolutionary forces driving the genetic trajectories of populations depends upon the sizes of these populations. However, rather than the actual number of individuals, the relevant quantity is the effective population size (N_e), defined by Wright (1931) as the size of an ideal population that would resist drift in the same way as the population under study. Ideal features include stable demography, an annual life cycle, panmictic reproduction (including the possibility of self-fertilization), and a Poisson distribution of fecundities.

Any departure from such assumptions may affect effective size (first discussed by Wright 1938; later reviewed by Lande and Barrowclough 1987; Crow and Denniston 1988; Frankham 1995). Iteroparity, for instance, may limit the action of genetic drift, because the same individuals contribute to the breeding

pool over several reproductive events. Similarly, a delayed maturity increases the ratio of effective breeding size to census breeding size by generating a stock of subadults that act as potential recruits (Nunney 1993). Other discrepancies from ideal settings may, conversely, increase genetic drift. Both theoretical and empirical studies have shown that, besides demographic fluctuations, the variance in reproductive success can drastically lower effective sizes (Kimura and Crow 1963; Frankham 1995; Hedrick 2005). Gonochorism (separate genders) has the potential to generate such a variance through biased sex ratios (Wright 1938). For the same reason, mating systems play a crucial role by affecting the distribution of fecundities, which may strongly depart from Poisson. Polygyny in particular has the potential to depress effective size by generating an important among-males variance in reproductive

output (Bateman 1948). Different kinds of polygyny (e.g., resource-defense, female-defense, dominance, etc. Emlen and Oring 1977) are likely to generate different impacts (Nunney 1993; Nunney and Elam 1994), a topic deserving empirical investigations. Lek-breeding, in particular, is expected to induce extreme levels of variance in male reproductive success (Emlen and Oring 1977).

Documenting the effects of mating systems on reproductive variance has relevance to both conservation and evolutionary biology. On the one hand, lowering effective size is expected to enhance extinction risk by increasing the rate of accumulation of deleterious mutations (Lande 1995), by impeding the fixation of beneficial alleles (Whitlock and Bürger 2004), or by limiting the evolutionary potential of populations to cope with environmental changes (Franklin 1980). On the other hand, mating systems may generate sex differences in the opportunity for selection (defined as the variance in relative fitness, Crow 1958), and thereby affect the rate of evolution of sex-specific traits (Arnold and Wade 1984a) as well as the level of diversity in genes with sex-specific inheritance (Wade and Shuster 2004). Partitioning this variance has the potential to shed light on sex-specific selective pressures stemming from the several components of fitness (including the sexual-selection component arising from the variance in mating success), and thereby to provide important insights into the evolution of sex-specific traits (Arnold and Wade 1984a,b).

In this article, (1) we delineate (Appendix A) how the partitioning of opportunity for selection proposed by Arnold and Wade (1984a,b) can be combined with Nunney's (1993) analysis of mating systems and life cycles to provide a general expression for effective size that accounts for sex-specific life-history and mating-system features, and (2) we illustrate this approach by partitioning sex-specific effective sizes in a population of the lek-breeding European treefrog (*Hyla arborea*). Pond-breeding amphibians living in seasonal environments are subject to strict time and space constraints that are bound to boost sexual selection in males. Studies on a variety of species have indeed shown that the variance in male mating success (defined as the number of matings obtained per breeding season) may reach very large values (e.g., Wagner and Sullivan 1992, our Table 2). This is true not only of the lek-breeding systems leaving a large scope for female choice, such as the one under study, but also of the male-dominance polygyny and scramble competition that characterize many *Bufo* and *Rana* species (Howard 1979; Arnold and Wade 1984a; Vieites et al. 2004). As a result, the effective number of breeding males may drop drastically, down to 1% of their census size (e.g., Scribner et al. 1997).

In this context, the reasons to focus on *H. arborea* are twofold. First, documenting the effect of its lek-breeding mating system on sex-specific effective sizes should help understanding the dynamics of genetic variance at sex-specific loci and the evo-

lution of its nascent sex chromosomes (Berset-Brändli et al. 2006, 2007, 2008a; Niculita-Hirzel et al. 2008). Although the proto-Y chromosome has been shown to display lower levels of standing genetic diversity than does the proto-X (Berset-Brändli et al. 2007), the difference seems fully accounted for by the numbers of copies per breeding pair (3X vs. 1Y), which appears at odds with the drop in male effective population size expected from its lekking habits (Berset-Brändli et al. 2007).

The second reason relates to conservation biology. Although widespread from the coasts of the Atlantic Ocean to the shores of the Black Sea, this species is threatened over a large part of its distribution by the anthropogenic fragmentation and isolation of its populations (e.g., Carlson and Edenham 2000; Edenham et al. 2000; Anderson et al. 2004; Arens et al. 2006), which enhances demographic stochasticity and genetic drift. The potential risk posed by this additional source of drift depends on the importance of other sources stemming from species-specific life-cycle and mating-system characteristics. The lek-breeding habits of *H. arborea* have the potential to drastically lower effective size, and thus to combine with habitat fragmentation to drive remnant populations into extinction vortices. How strong is this effect, and how does it compare to other effects stemming from other tree-frog life-history features (iteroparity and delayed maturity), remain open empirical questions.

Using field observations and genetic-assignment methods, we first document the mating system and sex-specific variance in annual breeding success (defined as the number of eggs laid or fertilized) in a tree-frog population, disentangling the effects of mating success and fecundity per mate on sex-specific effective sizes, to further our understanding of the dynamics of genetic variance at sex-specific loci. We then combine these data with life-history information to estimate the ratio of effective breeding size to census breeding size, to better quantify the threat imposed by genetic drift on tree-frog natural populations.

Material and Methods

ADULT SAMPLING

Our study site (Les Mossières; western Switzerland, 6°21.9'E, 46°32'N) consisted of four occupied neighboring ponds (approximately 4 × 4 m, 40 cm deep, 5–30 m distant from each other), isolated from the other closest occupied site by 1.5 km. We visited the site every two to four nights (depending on weather conditions) during the whole 2006 breeding season (22 nights in total, from April 14 to June 15), from dusk (between 2030 h and 2200 h) to about midnight. During each visit all calling males were captured and compared to a photographic data bank for identification (the black lateral line allows unambiguous individual recognition, Pellet 2007). Males already listed in the data bank were immediately released, whereas the others were photographed, measured

(snout to vent length), and sampled for buccal cells (using buccal swabs, Pidancier et al. 2003; Broquet et al. 2007) before release. All adult females encountered by chance in the process were also captured, photographed, measured, and similarly sampled for buccal cells before release. Buccal swabs were stored dry in a deep-freezer (-80°C) the same night for subsequent genetic analyses.

OFFSPRING SAMPLING

Ponds were further visited by daylight (from 0100 h to 0300 h) every four days, and searched for clutches. All clutches found were collected, and developmental stages at collection were recorded according to three classes (corresponding to Gosner (1960) stages 1–14, 15–18, and 19–20, respectively), to estimate laying date. All clutches were then reared in the laboratory until hatching, and the number of eggs (whether successfully hatched or not) from each clutch was recorded. Ten individual tadpoles from each clutch were further kept in the laboratory until a tiny portion of the velum (ca. 2 mm^2) was removed from the extremity of their tail, and deep-frozen (-80°C) for subsequent genotyping. Tadpoles were then kept a few more days in the laboratory until the tailfin regenerated, before being released into their pond of origin.

PARENTAL ASSIGNMENT AND BREEDING SUCCESS

DNA from buccal swabs and tail tips was extracted as described in Berset-Brändli et al. (2007). All adults and an average of 3.8 tadpoles per clutch were genotyped (i.e., four tadpoles per clutch, except for 12 clutches out of 131 in which fewer offspring survived long enough to be genotyped). This subsampling of four tadpoles per clutch was decided based on behavioral observations and previous extensive genetic analyses pointing to a complete absence of multiple paternities within clutches (T. Broquet, J. Jaquière, and N. Perrin, unpubl. data). All samples were genotyped for one mitochondrial sequence (control region 615 bp, Goebel et al. 1999) and seven nuclear microsatellite markers (Ha1–20, Ha1–25, Ha1–67, Ha1–103, Ha1–60, Ha5–201, and Ha5–22, Arens et al. 2000). The three last loci are sex-linked with sex-diagnostic alleles (Berset-Brändli et al. 2006, 2007), a feature providing much power for parental assignment. As power turned out to be nevertheless insufficient in some cases, two more autosomal microsatellite loci (HaD115 and HaB12, Berset-Brändli et al. 2008b) were genotyped in all adults and in one quarter of the clutches. Fathers were first assigned by excluding males with mismatching alleles (accounting for the presence of null alleles at low frequency in Ha 1–67, Berset-Brändli et al. 2008a). Then, given the genotypes of all father–offspring dyads per clutch, maternal genotypes were reconstructed, and compared to those of adult females caught during the breeding season. Maternal assignment was thus not performed by exclusion, because female sampling was not exhaustive.

For each adult j of sex i , mating success (P_{ij}) was measured as the number of successful matings, fecundity per mate (B_{ij}) as the number of eggs parented per mating, and breeding success (k_{ij}) as the total number of eggs parented. The sex-specific opportunities for selection on traits affecting the breeding success (I_i) were partitioned according to equations (A6) and (A7), to separate the components due to the variances in mating success (opportunity for sexual selection, $I_{p,i}$) and fecundity per mate ($I_{B,i}$), as well as to covariances (C_i). These values were used to evaluate sex-specific effective breeding sizes N_{bf} and N_{bm} according to equation (A3), with 95% confidence intervals (CI_{95}) obtained by bootstrapping data (number of eggs parented) 10,000 times in R 2.6.1 (R Development Core Team 2007). The effective breeding size of the population was estimated by combining sex-specific breeding sizes as in equation (A2), and compared to the genetic estimate obtained from autosomal markers using equation (A1), with CI_{95} obtained by bootstrapping genotypes 10,000 times. Finally, life-history information on age at maturity and adult survival was gathered from the literature and combined with our results to calculate effective size as in equation (A10).

Results

BREEDING SUCCESS

In total, 15 males were monitored as calling during the breeding season (from April 14 to June 15, Supporting Fig. S1), all of which were captured and genotyped. We did not find any noncalling males, but captured eight females. From the 138 clutches found (from April 21 to May 21), 131 were genotyped (one clutch was accidentally lost, four had a null hatching success, and two did not produce tadpoles surviving long enough to be genotyped), and assigned without ambiguity to one of the 15 calling males, with no evidence of multiple paternity. By contrast, only 55 clutches could be assigned to one of the eight females captured (from 1 to 20 clutches each). The remaining 76 clutches were assigned to 11 unsampled females, the genotypes of which were inferred from father–offspring genotypes. Thus, a total of 19 females and 15 males participated in the breeding season, and 22 mating pairs involving 19 females and 10 males formed over at least eight spawning nights (Supporting Fig. S1). Treefrogs have only one breeding season per year (from mid April to mid June), which we monitored intensively throughout. All clutches found in the four ponds during this season were collected and genotyped. Hence we are confident that all individuals that participated in the breeding season were identified. Some noncalling (satellite) males may have passed unnoticed, but should have been very few, because 40% of females were found by chance despite their discreet behavior and short presence time. If present, such satellite males did not contribute to reproduction (all clutches

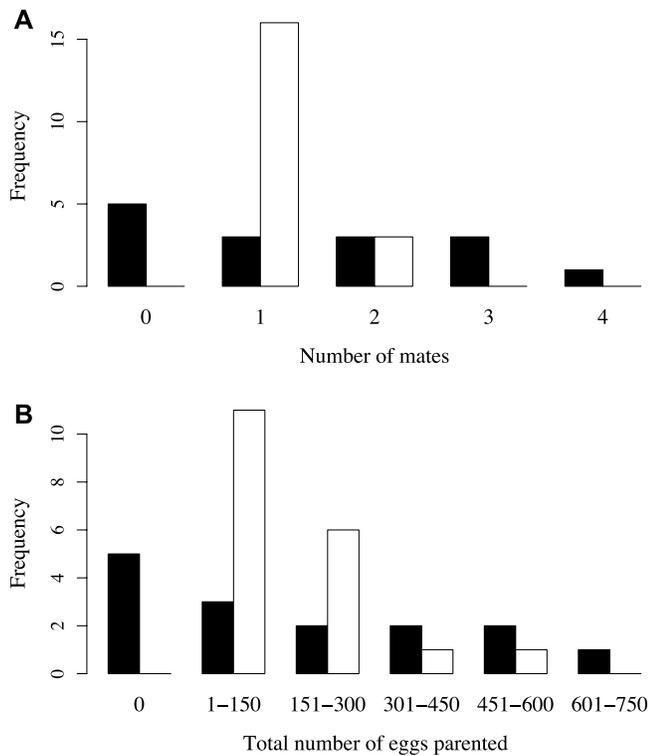


Figure 1. Distribution of male and female mating success (A), and breeding success measured as the number of eggs parented by individual (B). Male and female data are shown in black and white, respectively.

could be assigned without ambiguity to one of the calling males), and thus would not affect our estimates of effective breeding numbers.

Male and female mating success is shown in Figure 1A and in Supporting Table S1. Each female mated with a single male and laid her clutches during a single night, except for three females (females F2, F8, and F13, Supporting Fig. S1) that laid one to two small additional clutches (2–16 eggs) with a second male three weeks after the first batch (average $P_f \pm SD = 1.16 \pm 0.37$). Male mating success, by contrast, did not differ from a Poisson distribution. Five of the 15 calling males did not reproduce, whereas successful males mated with one to four females (average $P_m \pm SD = 1.47 \pm 1.31$, Fig. 1A). The number of clutches laid by females ranged 1–21 (amounting to 4–600 eggs, average $k_f \pm SD = 157.2 \pm 140$), whereas males fertilized 0 to 36 clutches each (0–658 eggs, average $k_m \pm SD = 199.1 \pm 225.1$; Fig. 1B and Supporting Table S1).

OPPORTUNITY FOR SELECTION AND EFFECTIVE POPULATION SIZE

Overall, the variance in relative breeding success (total opportunity for selection), calculated over the 34 adults identified on the breeding site, was about twice as large in males as in females: $I_m =$

1.28, $I_f = 0.75$ (Table 1). This difference stemmed mostly from the mating-success component, which was eight times higher in males than in females ($I_{p,m} = 0.80$; $I_{p,f} = 0.10$). By contrast, fecundity per mate was less variable in males ($I_{B,m} = 0.36$) than in females ($I_{B,f} = 0.87$), being averaged over several mating events. Interestingly, the covariance between mating success and fecundity per mate was slightly positive in males ($C_m = 0.12$), suggesting that individuals with a better mating success also had access to better partners, but negative in females ($C_f = -0.21$), because those that mated with a second male laid very few additional eggs. They had thus a similar breeding success, but spread over two matings instead of one. Discarding these second matings (so that the mating success and interaction terms vanished; $I_{p,f} = C_f = 0$) had negligible impact on the average and variance in female fitness ($k_f = 156.1$, $I_f = I_{B,f} = 0.76$), owing to their marginal contribution to female reproductive output.

The effective breeding pool was lower for males ($N_{bm} = 6.60$ [CI₉₅ 6.43–6.74]) than for females ($N_{bf} = 10.88$ [CI₉₅ 10.43–11.27]), due to both a lower census size ($N_m = 15$, $N_f = 19$), and the lower ratio of effective breeding size to census breeding size stemming from the mating system (0.44 vs. 0.57). Assuming equal sex ratio, the sex-specific variances in breeding success would reduce by half the ratio of effective to census breeding numbers (i.e., $\frac{N_e}{N_b} = \frac{1}{2}(\frac{1}{0.44} + \frac{1}{0.57}) = \frac{1}{0.50}$). By contrast, if breeding success were Poisson distributed in both sexes, the observed sex ratio would lower N_b by half-an-individual only (i.e., $N_b/N = 33.5/34$). Combining both effects (eq. A2) resulted in an effective number of breeders of 16.43 (with CI₉₅ [15.91–16.87] obtained from the CI₉₅ limits of sex-specific estimates), as compared to a census size of 34 (i.e., a ratio of 0.48). This expectation fits remarkably well with the loss of genetic variance from the breeding adult ($H_e = 0.68$, $n = 34$) to the offspring generation ($H_e = 0.66$, $n = 498$), which, according to equation (A1), points to an effective breeding pool of about 17 individuals (i.e., a ratio of 0.50).

Annual survival rates reported from the literature are relatively low: 0.20 for females (Friedl and Klump 1997) and 0.3 (Tester 1990; Pellet 2007) to 0.37 (Friedl and Klump 1997) for males. These values, based on mark–recapture studies, assume no breeding dispersal, and may thus underestimate true values. This is especially true for females, which are very discreet and visit breeding spots very briefly. Assuming an annual survival of $v = 0.30$, adults would reproduce over 1.43 seasons on average, enhancing the ratio of effective to census-breeding size from 0.483 to 0.486 (from eq. A10). For $v = 0.40$ and 0.50, values for A would be 1.67 and 2.0, and N_e/N_b would reach 0.487 and 0.488, respectively. Thus, whatever the true value, the effect of v on N_e appears to be marginal.

From published analyses of age-structure in breeding European treefrogs, males and females do not reach maturity before the age of two, and potentially three years (Tester 1990; Friedl

Table 1. Contribution of variance in mating success and in fecundity to total opportunity for selection in male and females treefrogs.

Source of variance in relative fitness	Symbol	Contribution to total opportunity for selection			
		♀		♂	
		Value	Percentage	Value	Percentage
Mating success (P_i)	$I_{P,i}$	0.10	13	0.80	63
Fecundity: number of eggs per mate (B_i)	$I_{B,i}$	0.87	115	0.36	28
Covariance between number of mates (P_i) and fecundity (B_i)	C_i	-0.21	-28	0.12	9
Breeding success: number of eggs (k_i)	I	0.75	100	1.28	100

and Klump 1997). Combined with an adult survival rate of $v = 0.3$, this delay in maturity appears to boost the ratio of effective breeding size to census breeding size from 0.486 to 0.825 or 1.165, for ages at maturity of two or three years, respectively (eq. A10). Given the strength of this effect, age at maturity appears to be a key parameter in the evaluation of tree-frog effective size, deserving precise quantitative estimation.

Discussion

The most striking result from the present study is the remarkably weak impact of lek-breeding habits on male effective population size. As developed below, this has consequences in terms of evolutionary biology (e.g., on the dynamics of sex-specific genes) as well as conservation biology (e.g., on the ability of natural populations to resist drift).

SEX-SPECIFIC BREEDING SIZES AND THE DYNAMICS OF SEX-SPECIFIC LOCI

As already pointed out, pond-breeding amphibians living in seasonal environments are subject to strict time and space constraints that are expected to boost sexual selection in males. Lek-breeding in particular was predicted by Emlen and Oring (1977) to induce extreme levels of variance in male reproductive success and thereby generate a particularly strong impact on effective population. In this context, the opportunity for male sexual selection

measured in our study population was surprisingly low ($I_{P,m} = 0.80$), compared with values reported from diverse amphibian species with a variety of breeding systems ($I_{P,m}$ may reach values up to 3 or 4 in other lek-breeding *Hyla* or *Bufo* species; see table 15 in Kluge 1981 and our Table 2 for reviews). Although opportunity for sexual selection was markedly lower in females ($I_{P,f} = 0.10$), the fecundity per mate was more variable ($I_{B,f} = 0.87$ vs. $I_{B,m} = 0.36$), so that the ratios of effective breeding size to census breeding size turned out to be surprisingly similar in both sexes ($N_{bm}/N_m = 0.44$; $N_{bf}/N_f = 0.57$).

Although unexpected given *Hyla*'s lekking habits, this result corroborates the patterns of diversity on *Hyla* sex chromosomes reported by Berset-Brändli et al. (2007, 2008a). Genetic diversity θ was found to be 2.66 times higher on the proto-X than on the proto-Y chromosomes, which appears incompatible with a strongly depressed male effective population size (Appendix B). To illustrate the potential of our partitioning of variance for investigations on the dynamics of sex-specific genes, we delineate in Appendix B how the sex-specific breeding sizes reported here can be combined with the diversity values observed on sex chromosomes to estimate the ratio of male-to-female mutation rate. Our estimate ($\alpha = 1.76$) appears to be in line with values ($\alpha = 2$) obtained from small mammals of similar sizes and generation times (Makova et al. 2004; Sandstedt and Tucker 2005; Handley et al. 2006).

Table 2. Opportunity for sexual selection on male mating success ($I_{P,m}$) in Amphibians.

Species	Mating system ¹	$I_{P,m}$	Reference
Strawberry dart-poison frog (<i>Dendrobates pumilio</i>)	resource-defense polygyny + polyandry ²	1.76 0.33–1.48	Pröhl and Hödl 1999 Pröhl 2002
Bullfrog (<i>Rana catesbeiana</i>)	scramble competition polygyny	1.38	Arnold and Wade 1984a
Gulf coast toad (<i>Bufo valliceps</i>)	lek polygyny	2.28–4	Wagner and Sullivan 1992
Barking treefrog (<i>Hyla gratiosa</i>)	lek polygyny	2.26–3.01	Murphy 1994
European treefrog (<i>Hyla arborea</i>)	lek polygyny	0.8	This study

¹Classification according to Sullivan et al. (1995).

²*Dendrobates pumilio* has a complex mating system involving both resource-defense polygyny (males defend breeding territories) and polyandry (females may mate several times).

Table 3. Ratio of effective to census number of breeders (N_b/N) in Amphibians.

Species	Mating system ¹	N_b/N	Method ²	Reference
Crested newt (<i>Triturus cristatus</i>)	lek polygyny ³	0.17–0.19	genet.	Jehle et al. 2001
Marbled newt (<i>Triturus marmoratus</i>)	lek polygyny ³	0.1–0.17	genet.	Jehle et al. 2001
Common toad (<i>Bufo bufo</i>)	scramble competition polygyny	0.005–0.012	genet.	Scribner et al. 1997
		0.034–0.04	genet.	Brede and Beebee 2006
Common frog (<i>Rana temporaria</i>)	scramble competition polygyny	0.33–0.365	genet.	Brede and Beebee 2006
		0.06–0.09	genet.	Schmeller and Merilä 2007
		0.07–0.17	demo.	Schmeller and Merilä 2007
European treefrog (<i>Hyla arborea</i>)	lek polygyny	0.48	demo.	This study
		0.5	genet.	This study

¹Classification according to Sullivan et al. (1995).

²For purposes of comparison, we report here only estimates of N_b based either on sex-ratio and variance in family size (“demo.”) or on genetic methods (“genet.”) applied within one generation (i.e., temporal change in allelic frequencies in parent vs. offspring samples).

³The mating system of *T. cristatus* and *T. marmoratus* has been classified as lek polygyny by Sullivan et al. (1995), based on the clustering behavior of displaying males. One may also refer to this system as “promiscuity” given that both males and females may mate with several partners during a prolonged breeding season.

PARTITIONING THE MATING-SYSTEM- AND LIFE-HISTORY COMPONENTS ON EFFECTIVE SIZE

From our results, lek-breeding seems to affect only weakly the ratio of breeding to census size in the study population. The ratio estimated here (0.48) fits remarkably well with the value (0.50) obtained from the observed loss of genetic variance on autosomal markers between the breeding adult and the offspring generation. Our partitioning showed this value to depend on the mating system much more than on the slightly biased census sex ratio. Hence, the variances in male mating success, in female fecundity, and in adult sex ratio can be ranked in a decreasing order regarding their effects on the effective numbers of breeders in the population under study.

This ratio lies above other values published for amphibians (range: 0.005 to 0.37; Table 3), very close to the average (0.46) calculated by Frankham (1995) over a large number of species regardless of mating systems, and not far from values (0.60) obtained from mating systems close to monogamy (Bouteiller and Perrin 2000). This limited impact, furthermore, seems largely compensated by other tree-frog life-history features. Delayed maturity, in particular, appears to boost effective size by generating a large pool of premature individuals that will be recruited later as breeders, so that instantaneous effective size approximately matches census breeding numbers. Our results thus suggest that the genetic drift imposed on European treefrogs by the anthropogenic reduction of their habitats is not severely enhanced by their lek-breeding habits.

This reasonably optimistic conclusion, however, has to be weighted by some cautionary notes. Although the effective number of breeders (N_b) could be precisely estimated owing to a quasi-exhaustive sampling of adults and their progeny, it must be emphasized that the effective population size (N_e) calculated

here does not account for a number of important features. First, male mating success is likely to be under female choice, and the lek-breeding behavior to be driven by good genes, given the nonresource-based nature of the tree-frog mating system. Any heritable component of male fitness should further enhance the long-term loss of genetic variance (Nei and Murata 1966; Kelly 2001; Nomura 2002). Second, the intensity of sexual selection might actually be reduced in small populations (such as the one under study), because females have a reduced choice of mating partners (“genetic compensation” sensu Ardren and Kapuscinski 2003). Our data might thus underestimate the loss of genetic variance occurring in larger populations. Third, the variance in offspring survivorship from egg to adult stage might not be random, but relate to genetic and environmental features, which would contribute to further reduce N_e (Rowe and Beebee 2004). Such effects were not included in the present study, which was limited to the estimation of annual breeding success (number of eggs parented) rather than lifetime reproductive success. Fourth, the temporal fluctuations in population size and disequilibrium dynamics that characterize *H. arborea* populations (Carlson and Edenham 2000; Pellet et al. 2006) are known to strongly depress long-term N_e (Frankham 1995), and should be accounted for in any global assessment of long-term effective sizes.

Finally, the role of connectivity must be emphasized. If isolated, the study population would lose genetic variance at a rate of $1/2 N_e \sim 2\%$ at each generation (notwithstanding all effects mentioned here above and not accounted for in the present study). Mutation rate is obviously not sufficient to maintain the current level of genetic diversity in this population ($H_e \sim 0.7$). *Hyla arborea* can be considered a vagile species (Vos et al. 2000), and immigration plays a crucial role in refuelling genetic diversity (Arens et al. 2006; C. Vogler, L. Berset-Braendli, J. Jaquière, and

N. Perrin, unpubl. ms.). The preservation of well-connected population networks is undoubtedly critical for the long-term persistence and maintenance of genetic diversity in local tree-frog populations.

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Appendix A

OPPORTUNITIES FOR SELECTION, LIFE CYCLES, AND EFFECTIVE SIZES

Sex-specific patterns of reproduction and effective number of breeders

The amount of genetic variance maintained in local populations is set by the dynamic equilibrium between the evolutionary forces that tend to lower it (drift and selection) and those that tend to restore it (dispersal and mutation). Notwithstanding mutations, genetic variance is lost during a breeding episode as

$$H_{e,t+1} = \left(1 - \frac{1}{2N_b}\right) H_{e,t}, \quad (\text{A1})$$

where $H_{e,t}$ measures heterozygosity in the parental population and $H_{e,t+1}$ in the offspring generation (before dispersal). N_b is the effective number of breeders, which depends on the effective numbers of breeding males N_{bm} and females N_{bf} (Kimura and Crow 1963; Falconer 1986, p. 64; Hedrick 2000, p. 250):

$$\frac{1}{N_b} = \frac{1}{4} \left(\frac{1}{N_{bm}} + \frac{1}{N_{bf}} \right). \quad (\text{A2})$$

These effective numbers N_{bi} ($i = f, m$) can be expressed as a function of census size (N_i) as well as mean (k_i) and variance (V_i) in reproductive success (Kimura and Crow 1963; Lande and Barrowclough 1987; Hedrick 2000, p. 250):

$$\frac{1}{N_{bi}} = \frac{k_i + V_i/k_i - 1}{N_i k_i - 1}. \quad (\text{A3})$$

Note that equation (A3) can be written $\frac{1}{N_{bi}} = \frac{1+I_i-1/k_i}{N_i-1/k_i}$, where $I_i = \frac{V_i}{k_i^2}$ measures the variance in relative fitness (or, equivalently, the difference in relative fitness between breeding adults and the average parental generation). Because variance in fitness stems from both drift and selection, this value sets the upper boundary for the action of selection, and is thus referred to as an “opportunity for selection” (Bateman 1948; Crow 1958, 1962; Arnold and Wade 1984a; Shuster and Wade 2003, chapter one). In our case, k_i represents an average breeding success in terms of egg number, which is large enough that $1/k_i$ can be safely neglected, so that (A3) becomes

$$\frac{1}{N_{bi}} \approx \frac{1 + I_i}{N_i} \quad (\text{A4})$$

Partitioning the opportunity for selection

The breeding success k_i can be partitioned into two main components, namely the number of mates (P_i) and the fecundity per

mating (B_i)

$$k_i = P_i B_i, \quad (\text{A5})$$

Thus, the opportunity for selection on traits affecting the breeding success (I_i) can be similarly partitioned into two main components, corresponding to the variances in relative mating success ($I_{P,i}$) and fecundity per mate ($I_{B,i}$), respectively (Arnold and Wade 1984a,b)

$$I_i = I_{P,i} + I_{B,i} + C_i, \quad (\text{A6})$$

where C_i represents the covariance terms between fitness components P_i and B_i . These components can be estimated following Arnold and Wade (1984a, eqs. 14 and 15):

$$I_{P,i} = \frac{\sum_j (p_{ij})^2}{N_i} - 1, \quad (\text{A7a})$$

$$I_{B,i} = \frac{\sum_j p_{ij}(b_{ij})^2}{N_i} - 1, \quad (\text{A7b})$$

where p_{ij} measures the relative mating success (i.e., individual value divided by population average), and b_{ij} the relative fecundity per mating, for individual j of sex i (see Arnold and Wade 1984a for more details, and refer to their appendix for the decomposition of the covariance term C).

Plugging (A6) into (A4) and (A2) provides a measure of the effective number of breeders in terms of sex-specific census sizes and opportunities for selection

$$\frac{1}{N_b} \approx \frac{1}{4} \left(\sum_{i=m,f} \frac{1 + I_{P,i} + I_{B,i} + C_i}{N_i} \right), \quad (\text{A8})$$

which allows teasing apart the contributions of the several sex-specific components of breeding success to the loss of genetic variance.

Overlapping generations and delayed maturity

In many species, generations overlap and age at maturity (M) exceeds one year. Both features are expected to affect the ratio of effective breeding size census breeding size (Nunney 1993; Nunney and Elam 1994). Assuming the patterns of fecundity (both average and variance) to remain constant after maturity, then equation (A8) becomes

$$\frac{1}{N_e} \approx \frac{1}{4T} \left(\sum_{i=m,f} \frac{A_i (1 + I_{A,i}) + I_i}{N_i} \right), \quad (\text{A9})$$

where A_i and $I_{A,i}$ are respectively the average and relative variance in adult life span, and T is generation time. This measure of effective size (N_e) differs from N_b in that it also accounts for the

preadult individuals (aged less than M) that will later be recruited as breeders, and for the possible survival and ensuing reproduction of individuals over several years. Assuming a constant adult survival rate v (i.e., type 2 survivorship), then $A_i = 1/(1 - v_i)$ and $I_{A,i} = v_i$. The generation time is calculated as $T = A + M - 1$, where $A = (A_m + A_f)/2$ is the average adult life span and M the average age at maturity. Our equation (A9) corresponds to equation (A2) in Nunney (1993) and to the “minimal model” equation in the Appendix of Nunney & Elam (1994).

Both features potentially increase the ratio of effective breeding size to census breeding size (N_e/N). Increasing adult survival (v), on the one hand, allows the same individuals to reproduce over several years, reducing the action of genetic drift. Increasing age at maturity (M), on the other hand, generates a pool of nonbreeding sub-adults that act as potential recruits. As shown by Nunney (1993, eq. 22), $N_e/N_e' = T/A$, where N_e' is the effective size of a population that is identical except that juveniles take one season to mature instead of M .

Plugging (A6) into (A9) thus provides a general expression for effective size that teases apart all main sex-specific life-history contributions to the loss of genetic variance:

$$\frac{1}{N_e} \approx \frac{1}{4T} \left(\sum_{i=m,f} \frac{A_i (1 + I_{A,i}) + I_{P,i} + I_{B,i} + C_i}{N_i} \right). \quad (\text{A10})$$

Appendix B

SEX-SPECIFIC EFFECTIVE SIZES AND THE DIVERSITY OF SEX-LINKED GENES

Genetic diversity on the X chromosome (θ_X) is expected to exceed that on the Y chromosome (θ_Y) for several reasons. Assuming mutation–drift equilibrium and notwithstanding processes stemming from the nonrecombining nature of the Y chromosome, the ratio θ_X/θ_Y should depend on effective sizes and mutation rates as $\theta_X/\theta_Y = N_b^X \mu_X / N_b^Y \mu_Y$, where $N_b^X = \frac{9N_{bm}N_{bf}}{2N_{bf} + 4N_{bm}}$ and $N_b^Y = \frac{N_{bm}}{2}$ measure the effective sizes of the X and Y sex chromosomes, whereas μ_X and μ_Y measure their mutations rates. A ratio value of $\theta_X/\theta_Y = 3$ is thus expected if sexes have identical breeding sizes ($N_{bm} = N_{bf}$) and chromosomes identical mutation rates ($\mu_X = \mu_Y$). Higher ratios may occur if males have lower breeding sizes ($N_{bm} < N_{bf}$), whereas lower ratios may occur if males have higher mutation rates (i.e., if the ratio of male to female mutation rate, $\alpha = \frac{2\mu_Y/\mu_X}{3 - \mu_Y/\mu_X}$, exceeds one).

Berset-Brändli et al. (2007) estimated the θ_X/θ_Y ratio to be 2.52 in *H. arborea*. A very similar ratio ($\theta_X/\theta_Y = 2.66$) can be computed from the expanded dataset of Berset-Brändli et al. (2008a). From our present estimates of breeding sizes ($N_{bm}/N_m = 0.44$ and $N_{bf}/N_f = 0.57$), and assuming equal sex-ratios ($N_m = N_f$), the ratio of sex-chromosome effective sizes

becomes $N_b^X/N_b^Y = 3.54$. Combined with $\theta_X/\theta_Y = 2.66$, this provides a μ_Y/μ_X ratio of 1.4, pointing to a value of $\alpha = 1.76$ for the ratio of male to female mutation rate, in line with values

($\alpha = 2$) obtained from small mammals of similar sizes and generation times (e.g., Makova et al. 2004; Sandstedt and Tucker 2005; Handley et al. 2006).

Supporting Information

The following supporting information is available for this article:

Figure S1. Timetable of adult activity.

Table S1. Reproductive success of 19 female and 15 male treefrogs

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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