Adaptive changes in sexual signalling in response to urbanization

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Urbanization can cause species to adjust their sexual displays, because the effectiveness of mating signals is influenced by environmental conditions. Despite many examples that show that mating signals in urban conditions differ from those in rural conditions, we do not know whether these differences provide a combined reproductive and survival benefit to the urban phenotype. Here we show that male túngara frogs have increased the conspicuousness of their calls, which is under strong sexual and natural selection by signal receivers, as an adaptive response to city life. The urban phenotype consequently attracts more females than the forest phenotype, while avoiding the costs that are imposed by eavesdropping bats and midges, which we show are rare in urban areas. Finally, we show in a translocation experiment that urban frogs can reduce risk of predation and parasitism when moved to the forest, but that forest frogs do not increase their sexual attractiveness when moved to the city. Our findings thus reveal that urbanization can rapidly drive adaptive signal change via changes in both natural and sexual selection pressures.

One of the most prominent characteristics of the Anthropocene is the rapid turnover of the natural world into human-dominated landscapes[1,2]. This turnover is particularly visible in vast urbanized areas where millions of people rely on residential and industrial spaces, which are interconnected by transportation lines, such as roads, railways, flights and shipping routes[3–5]. The strongly different environmental and climatic conditions in cities compared to rural areas affect many species and their ecological communities[5–7]. Consequently, the rapid expansion of urban areas and their interconnected structures forces many species to adjust or move to other areas[7,8].

Urbanization is likely to impose strong selection on animal communication, as effective production, transmission and perception of signals is heavily influenced by environmental conditions[9], such as differences in noise or light pollution, or food availability[10–15]. Urbanization may also affect the abundance of predators and parasites that tune in on communication signals, thereby indirectly altering the selection pressures that operate on signallers[16–19]. Given the divergent selection regimes, it is not surprising that urban animals often sound, look and behave differently compared to their nonurban conspecifics. Unfortunately, for many cases of urban-dependent changes in signals, we cannot assess whether these differences are adaptive, because we lack a comprehensive understanding of the associated fitness consequences[20–23].

Most studies of communication in urbanized areas have focused on the production of acoustic signals that are influenced by anthropogenic night lighting and noise pollution[24–26]. Birds, frogs and grasshoppers have repeatedly been shown to sing or call differently in noisy urban areas compared to quieter and darker rural areas[27–30]. However, urban-altered signalling can only be adaptive if the balance between mate attraction and risk of predation and parasitism is improved or maintained. Few studies have addressed the combined sexual and natural selection pressures and we thus lack a good

understanding as to whether urban-altered signalling reflects an adaptive response[31–33].

We studied the communication system of the túngara frog (*Physalaemus pustulosus*), a Neotropical species that gathers at night in puddles to call and attract females[34]. Males can increase the complexity as well as the attractiveness of their calls to females by adding elements called 'chucks'[34–36]. Sexual selection for these more elaborate displays is, however, counterbalanced by natural selection imposed by predators and parasites, as bats and midges also preferentially attack males that produce more complex calls[37–40]. Notably, the communication system of the túngara frog is affected by conditions that are associated with increased urbanization. Males alter the rate, complexity and amplitude of their calls in response to experimental noise exposure[41], whereas the preference of females for males using complex calls may be influenced by light levels[42]. Signal eavesdroppers, on the other hand, seem mainly negatively impacted by noise and light pollution[43,44], although predatory bats may be able to adapt by switching to other sensory modalities[43].

We assessed whether male túngara frogs have altered their signalling behaviour in response to urbanization and we aimed to understand how this signal change relates to differences in sexual and natural selection pressures. First, we recorded males in eleven paired urban and forest sites and related variation in their mating display to variation in noise and light conditions. Second, we estimated differences in the strength of sexual selection and natural selection by broadcasting male calls and by quantifying the number of female frogs, frog-eating bats and frog-biting midges that were attracted by these calls in both urban and forest populations. Third, we tested urban and forest females for their preference for urban and forest sexual signals in a phonotaxis experiment. Finally, we tested the extent of signal flexibility by translocating males from five urban and five forest populations to two urban and two forest sites. The outcome of these experiments allowed us to assess whether the

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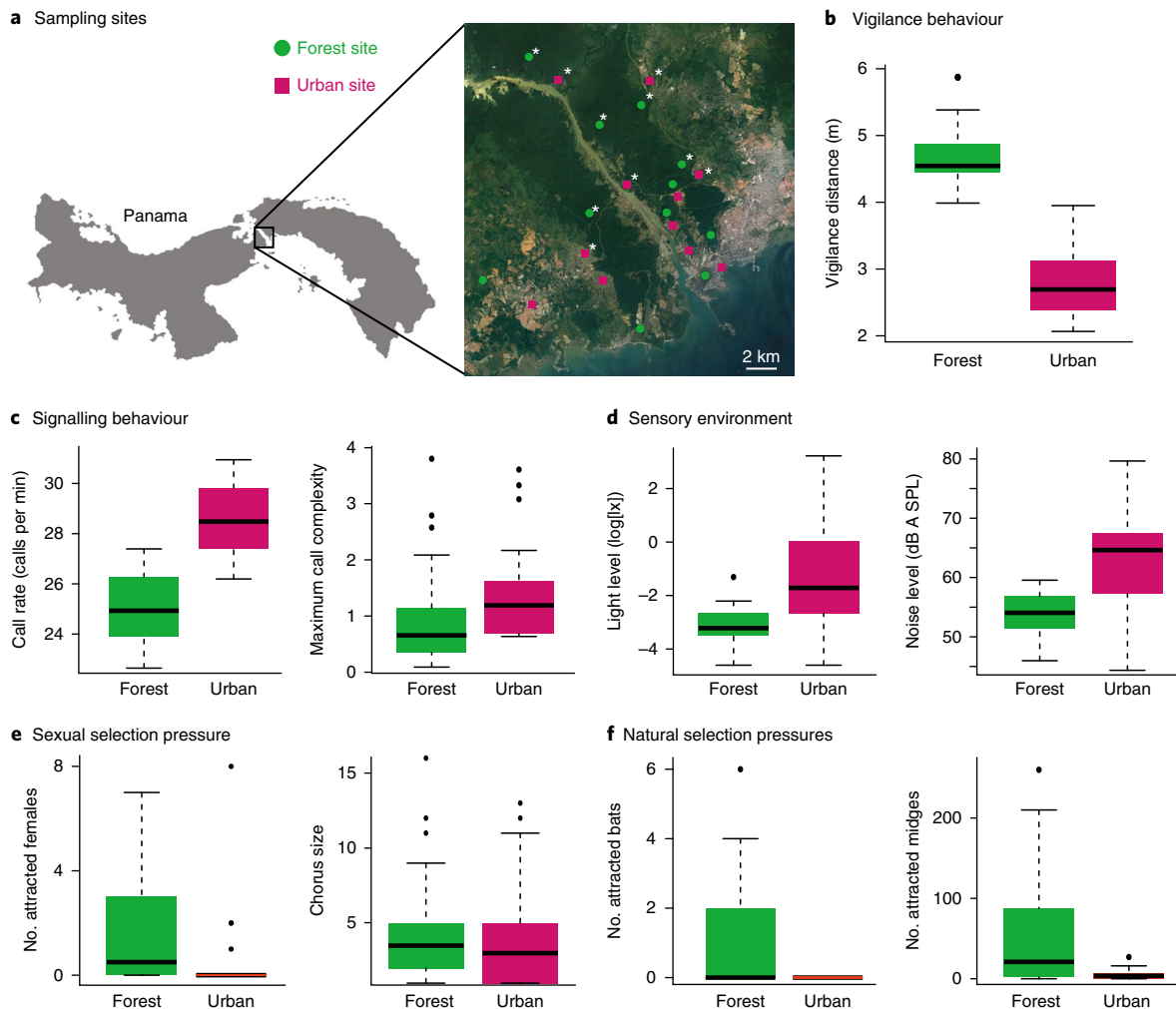
Fig. 1 | Differences in calling behaviour and selection pressures between urban and forest populations. a, Maps show the locations of the 22 forest and urban sites on both sides of the Panama Canal (see Supplementary Table 2 for coordinates). White asterisks indicate populations used for the translocation experiment and for the recordings of stimuli for the female choice experiments. **b,c**, Differences in calling behaviour. Urban males are easier to approach (**b**, $n=83$) and call at higher rate (**c**, $n=93$) and with more complexity (**c**, $n=98$) compared with forest males. **d–f**, Differences in selection pressures. **d**, Noise and light levels are higher in urban compared to forest sites ($n=100$). **e**, In urban areas, fewer females are attracted to call playback ($n=43$), although the chorus size of males is equal between habitats ($n=98$). Urban males consequently experience higher competition for mating opportunities. **f**, Rates of predations and parasitism are lower in urban sites compared with forest sites ($n=44$). Box plots depict the median, first and third quartiles and 1.5× interquartile range of model estimates (**b,c**) or raw data (**d–f**). See Supplementary Table 1 for model outputs.

urban-induced change in the sexual phenotype reflects an adaptive response by comparing the success of an individual to obtain mates versus the risk of predation in both urban and forest areas.

Results

Urban males display more elaborate mating calls. We recorded male túngara frog calls in 11 urban and 11 forest sites (Fig. 1a) and found that calling behaviour and signal design were different between the two habitat types. Forest males were quicker to cease calling compared to urban males when approached by a human observer (general linear mixed model (GLMM), $n=83$, $\chi^2=10.13$, d.f. $=1$, $P<0.001$; Fig. 1b and Supplementary Table 1). Urban males were found to call at higher rates (GLMM, $n=93$, $\chi^2=5.69$, d.f. $=1$, $P=0.017$) and with greater call complexity (GLMM, $n=98$, $\chi^2=10.01$, d.f. $=1$, $P=0.002$) compared to forest males (Fig. 1c and Supplementary Table 1). Additionally, urban males were smaller in snout–vent length (GLMM, $n=260$, $\chi^2=14.42$, d.f. $=1$, $P<0.001$), but similar in mass (GLMM, $\chi^2=0.43$, d.f. $=1$, $P=0.51$), compared to forest males (Supplementary Table 1). Urban sites were characterized

by a distinctly different sensory environment, as noise and light levels were higher (GLMM, $n=98$; $\chi^2=44.25$, d.f. $=1$, $P<0.001$ and $\chi^2=30.91$, d.f. $=1$, $P<0.001$, respectively), and canopy cover was lower ($\chi^2=161.87$, d.f. $=1$, $P<0.001$) compared to forest sites (Fig. 1d and Supplementary Table 1). Differences in sensory environment were mainly due to presence of streetlights and low-frequency traffic noise in urban areas[41]. These findings suggest that the altered sensory conditions may have caused urban males to adjust their calling behaviour directly and/or affected intended and unintended signal receivers, thereby causing a shift in sexual and natural selection pressures imposed on urban signallers.

Variation in mating displays is related to divergent selection pressures. We broadcast a standardized túngara frog call in our 11 urban and 11 forest sites on two different nights per site, monitored approaching females and bats with a video-surveillance setup and collected midges with a custom-built trapping system (see Methods). In urban sites, the calls attracted fewer females (GLMM, $\chi^2=4.38$, d.f. $=1$, $P=0.038$), bats (GLMM, $\chi^2=15.56$, d.f. $=1$,
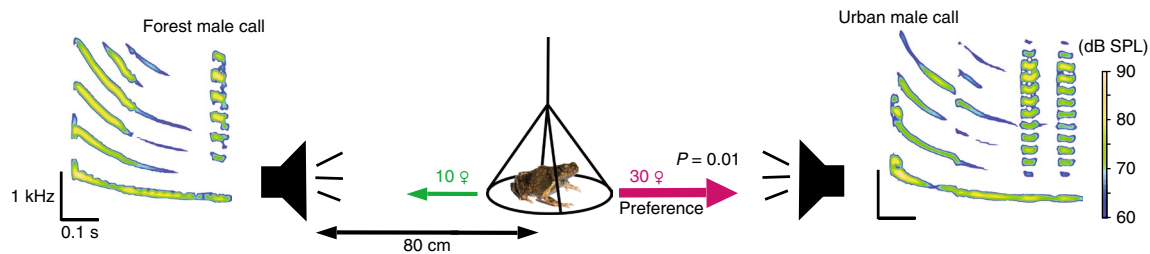
Fig. 2 | Females prefer urban over forest males. The setup and outcome of a mate-choice experiment in which females from urban and forest locations were given a choice to approach a speaker that broadcasted the call of either a forest (left spectrogram, see Supplementary Data 1 for audio file) or urban male (right spectrogram, see Supplementary Data 2 for audio file; note differences in chucks between the calls). Females were kept under a funnel and released after 2 min playback of a stimulus set that broadcasted alternating urban and forest calls. In total, 30 out of 40 tested females approached the speaker that broadcasted the urban call, which had on average 0.8 more chucks.

$P < 0.001$) and midges (GLMM, $\chi^2 = 15.05$, d.f. = 1, $P < 0.001$) compared to forest sites (Fig. 1e,f and Supplementary Table 1). The chorus densities of males did not differ between urban and forest sites (GLMM, $n = 98$; $\chi^2 = 0.51$, d.f. = 1, $P = 0.32$; Fig. 1e). Thus, the lower number of females, bats and midges that responded per calling male suggests that sexual selection pressure was higher and natural selection pressures that were operating on the signal were lower in urban areas compared to forest areas.

Urban males are more attractive to females. To assess whether the urban phenotype is adaptive in terms of mate attraction, we carried out a phonotaxis experiment with females collected from an urban and a forest location. We tested these females for their preference to approach one of two speakers that broadcasted calls of an urban or forest male from opposite sides of a sound-attenuating chamber. We captured 20 males at five different urban and five different forest sites (Fig. 1a) and recorded their calls in the laboratory under controlled light and sound conditions. The calls were broadcasted antiphonally (thus adjusting to the same call rates to avoid overlap between the calls) while keeping naturally recorded variation in amplitude profile and call complexity (see Methods). Stimuli from urban males that were recorded under common garden conditions had an average complexity of 1.7 ± 0.68 (mean $\pm$ s.d.), whereas forest male stimuli had an average complexity of 0.9 ± 0.74. Furthermore, urban stimuli had, on average, chucks that were similar in amplitude to that of the whine ($0.1\,\mathrm{dB} \pm 6.4$ (mean $\pm$ s.d.) relative difference in peak amplitude), whereas forest stimuli had, on average, chucks that were lower in amplitude than the whine ($-1.5\,\mathrm{dB} \pm 8.3$). Females were only tested once and strongly preferred the urban over the forest male call, irrespective of the female's origin (binomial test; $n_{\mathrm{females}} = 40$; $n_{\mathrm{stimulus\ pairs}} = 10$; probability to approach an urban male $= 0.75$; $P = 0.002$; Fig. 2). These data suggest that the urban phenotype is driven by a combination of increased competition over females as well as relaxed predation or parasitic pressure in urban environments.

Urban males display a broader range of sexual phenotypes. To test whether males can flexibly alter their signalling depending on environmental conditions, we conducted a translocation experiment. We collected 112 males from 5 different forest and 5 different urban populations (Fig. 1a). We transferred the frogs for at least four consecutive nights to one of four mesocosms that were located in either a forest or an urban environment (Fig. 3a). The mesocosms were located away from major roads, or other major sources of anthropogenic noise, so that abiotic conditions mainly differed in the amount of canopy cover and light pollution. The mesocosms were covered in mosquito mesh to keep frogs inside, and to prevent bat predation and midge parasitism. The translocation environment had a strong effect on male call rate (GLMM, $n = 16$ mesocosms;

6–8 males per mesocosm; four recording nights; measured at the start of call bout: $\chi^2 = 20.78$, d.f. = 1, $P < 0.001$; Fig. 3b) as well as on male call complexity (GLMM, $\chi^2 = 10.45$, d.f. = 1, $P = 0.001$; Fig. 3c). Overall, males called at higher rates and with more complex calls in urban compared to forest mesocosms. The population of origin had no overall effect on the calling behaviour of males (GLMM, call rate: $\chi^2 = 0.68$, d.f. = 1, $P = 0.41$; mean call complexity: $\chi^2 = 1.30$, d.f. = 1, $P = 0.25$), but its effect depended on the translocation environment (GLMM, interaction effect on call rate: $\chi^2 = 3.85$, d.f. = 1, $P = 0.049$; interaction effect on call complexity: $\chi^2 = 7.28$, d.f. = 1, $P = 0.007$). Urban males placed in a forest environment decreased call complexity compared to urban males placed in an urban environment (post hoc independent contrast; $z = -4.33$, $P < 0.001$; Fig. 3c), whereas a forest male placed in an urban environment did not change call complexity compared to a forest male placed in a forest environment ($z = 0.94$, $P = 0.74$; Fig. 3c). In the urban environment, urban males had, on average, more complex calls at the start of a call bout as well as during the peak of chorus activity and had a higher maximum number of chucks compared to forest males. These results show that urban frogs are better able to adjust their signalling to ecological conditions, probably using abiotic cues such as differences in sound and light environment. This higher level of signalling flexibility allows urban males to display the phenotype that best matches the balance between multiple selection pressures in both urban and forest environments.

Discussion
We found that male túngara frogs from urban areas call at a higher rate and with more complexity than males from forest areas. Differences in signalling were related to differences in the selection pressures imposed on the signallers in both environments; urban males experienced more competition for mates, but had a lower risk of predation and parasitism compared to forest males. These changes in biotic selection pressures between urban and forest areas could reflect differences in either receiver abundances or signal detection. Notably, when we recorded males under the same noise and light conditions, the urban calls were more attractive to females than the forest calls. These results suggest that the change in signalling associated with the urban phenotype was selectively favoured through increased benefits from female attraction and decreased costs of unwanted eavesdroppers. Furthermore, translocating males to urban and forest environments revealed that urban males can rapidly change their call rates and complexity, which in turn matches the risk of predation and parasitism, but that forest males do not adjust their call complexity in an urban environment. Thus, urban and forest males may differ in their response to changes in habitat (that is, differ in their reaction norm as shown by the significant interaction terms), suggesting that forest males may be outcompeted by urban males in the urban environment, but not the other way around.
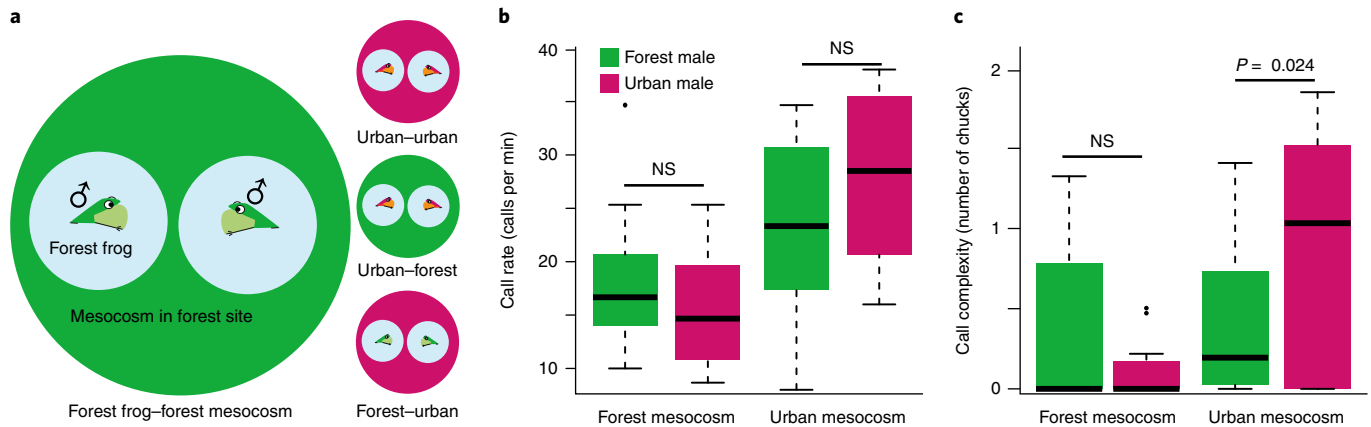
Fig. 3 | Forest frogs do not increase call complexity in the urban environment. Results from a translocation experiment in which we placed frogs from forest and urban populations in a forest or urban environment. **a**, Experimental setup, including one larger example for easy reference. **b**, Both urban and forest frogs can adjust their call rate to the environment within a few days. **c**, Urban frogs can adjust their average call complexity to the forest environment, but forest frogs do not adjust their complexity to the urban environment. See main text for comparisons between mesocosms. Raw data of calls recorded at the start of a calling bout are shown ($n = 16$ mesocosms, $n = 112$ males). Call behaviour at peak intensity shows similar patterns. NS, not significant.

There are several underlying mechanisms, which are not mutually exclusive, that may be responsible for the divergence in sexual signalling. Experimental evidence has demonstrated that túngara males can flexibly adjust call rate, amplitude and complexity in response to changes in their social[45], dietary[46] or sensory environment[41], and that some of these responses can be mediated by changes in circulating hormone levels[47]. Urban and forest males could thus differ in their hormone physiology, as a result of developmental or heritable differences[48,49]. Physiological differences would also explain the observed pattern in vigilance behaviour; urban males were less vigilant than forest males, presumably because of decreased predation risk or increased levels of human disturbance[49,50]. The level of vigilance is thought to be under strong control of circulating hormone levels and is often traded against other activities, including communication[49]. Alternatively, the morphological structures, such as lung capacity[51], vocal sac size[51,52] and the size of the fibrous mass attached to the vocal cords[35] may differ between urban and forest frogs, although this seems to be less likely, because urban males would also have to compensate for their, on average, smaller size.

We argue that urban signallers have a more flexible phenotype that may have important eco-evolutionary consequences. Urban males in our study produce a more elaborate and attractive mating display, which does not seem to come at an increased cost imposed by eavesdroppers, as urban males are able to quickly adjust their display to match the ecological requirements of the forest. Urban individuals may also be more flexible or show better adaptation of other behavioural, non-communicative traits[48,53]—such as risk-taking or problem-solving behaviours—compared to forest individuals. Notably, high behavioural flexibility across different species has been linked to successful colonization of urban areas in comparative studies[49,54]. The urban environment thus seems to select for individuals that are more flexible[55], less risk aversive[50] and more attractive than their rural counterparts. Our data suggest that these urban phenotypes have a reproductive advantage over the forest phenotypes in urban areas, whereas in forest areas none of the phenotypes has an advantage in terms of mate attraction, or predator and parasite avoidance. The most important driver of this selective advantage seems to be the absence of predators. Predators tend to be the first species that disappear from polluted[56] or fragmented sites[57], which would suggest that urban males also outcompete forest males in degraded forest habitats. The fact that urban phenotypes seem to have a higher-than-average flexibility at no apparent additional

costs may even result in the complete replacement of the forest male phenotype, depending on specific ecological factors, such as population sizes and dispersal rates.

In conclusion, we show that on the one hand, urban males experience an increase in the strength of sexual selection and a decrease in the strength of natural selection on the other hand. Increased signal conspicuousness through more complex calls thus provides a selective benefit to urban males in terms of mate attraction, without incurring the cost of increased predation or parasitism. Previous studies of urban-associated conditions have argued that changes in sexual signalling can either be adaptive or maladaptive[23,58]. Our results provide evidence that urban males can display an adaptive phenotype that may have resulted either as a direct response to changes in the environment or as an indirect response to changes in the sexual and natural selection pressures that operate on the phenotype. Human-dominated landscapes, such as cities, typically alter a whole suit of environmental selection pressures and our study demonstrates that species can adaptively respond to these changes, which has important eco-evolutionary consequences for population structure and community composition.

Methods

Study sites and species. We focused on the túngara frog (*P. pustulosus*), a species that is common in both urban and forest habitats. In the rainy season (May–December), males of this species gather at night and call to attract females[34]. Males can be found in a wide variety of water bodies, such as puddles on the forest floor, ditches next to roads or drainage systems in urban areas. The call of a male always starts with a down-sweeping harmonic element known as whine after which a male can add up to seven amplitude-modulated elements known as chucks[36]. The production of these chucks makes the call more complex and is known to be under both sexual and natural selection by intended and unintended receivers[37–40]. When given a choice, females prefer more complex over simple calls. Similarly, predatory bats (*Trachops cirrhosus*) and parasitic midges (*Corethrella* spp.) eavesdrop on the call and also have a strong preference to attack males that make chucks over males that do not[37,38].

We collected data in the field from May to July 2016 and in May 2018 in the canal area of Panama. We sampled male frog behaviour and (a)biotic conditions in 22 different populations (11 urban and 11 forest) on both sides of the Panama Canal (Supplementary Table 2), as well as on both sides of the Chagres river. We used a paired design in which the distance between an adjacent forest and urban site was always smaller than between other sites of the same habitat type. Distances between paired sites were 0.6–7.0 km. Sites with various degrees of urban development, from small towns (Gamboa) to more developed areas in Panama City, were considered urban populations. Additionally, we brought male túngara frogs to Gamboa, Panama, for laboratory recordings as well as for a translocation experiment. In May 2017, we conducted phonotaxis experiments with females collected from Gamboa (urban site), as well as the nearby Soberanía National Park

(forest site). Frogs were brought to the laboratory in small plastic containers and were kept in a plastic cooler in between recordings or experiments. Males and females were toe-clipped for individual recognition after the experiment and their weight and snout–vent length were measured. All individuals were released where they were collected on the same night, or after one week for the males used in the translocation experiment. All experiments with frogs were licensed and approved by the STRI (IACUC permit: 2015-0618-2018) and the Autoridad Nacional del Ambiente de Panama (SE/A-38–17; SE/A-2-16).

Field recordings. Calling males were recorded with a directional microphone (Sennheiser ME66) attached to a field recorder (Marantz, PMD660) from a distance of 0.5–1 m. Males were approached and recorded in a standardized way to score their vigilance behaviour. One of the researchers would slowly approach a calling frog and place a small tripod with a microphone at ~1 m from the edge of the water body from which the frog called. Vigilance distance was the distance at which a male ceased calling when approached. We did not score vigilance distance when males were calling from confined spaces from which they could not observe us approaching. Males were recorded for several minutes after which we scored chorus size by counting the number of calling males in a radius of ~5 m. Additionally, we recorded noise and light levels and scored vegetation density and canopy cover at the position of calling frogs at all of our recording sites. Noise levels were measured with a sound pressure level (SPL) meter (Voltcraft SL-100, A-weighted, set to fast window, max intensity) pointing in four different directions. Light levels were obtained in a similar way with a lux meter (HT Instruments HT309). The four measurements were averaged per call site. Vegetation density and canopy cover were estimated by taking a photograph and using the magic wand tool in Adobe Photoshop CS5. In 2018, we obtained pilot data on air, water temperature and humidity at 13 locations using a Sper Scientific environmental meter 850070 at calling sites in a subset of the study sites that we visited in 2016. Puddles with a minimum of three calling males were visited at night between 20:00 and 24:00. Water temperature was measured at the position of calling males using a type K thermocouple probe. For each puddle, the water temperature for three males was averaged. In order to compare temperature and humidity between urban and rural sites, we used a generalized mixed model with sites, date and time as random effects. These data revealed that air temperature, water temperature and humidity did not vary significantly between urban and forest puddles ($n = 54$, $P = 0.56$; $n = 54$, $P = 0.19$; and $n = 41$, $P = 0.53$, respectively; Supplementary Table 3).

Quantifying selection pressures in the field. We conducted field playback experiments at all of our sampling sites to assess mate attraction, as well as rates of parasitism and predation for a calling male frog. We broadcast a synthetic signal[59] that consisted of a whine plus one chuck at 82 dB (SPL reference to 20 μ Pa, measured at 1 m with the SPL meter using a 1 kHz reference tone with the same amplitude level as the signal) at 0.5 calls s^{-1} from two different locations per site. This synthetic signal reflects average measurements of a large set of acoustic parameters and is based on recordings from a large set of males collected from urban and forest locations in the vicinity of Gamboa. We used a custom-built playback setup (USB soundboard connected to a Visaton EX 60S shaker attached to a Plexiglas circular plate with a diameter of 20 cm, amplified using a 18 V Kemo amplifier and 18 V lithium battery) to broadcast calls for 1 h between 19:00 and 20:30. The speaker platform was covered in sticky foil (Tanglefoot Tangle-Trap Insect Trap Coating, Contech Enterprises) to collect acoustically orientating insects. We monitored the presence of female frogs and frog-eating bats with two cameras (Panasonic HX-A1) using infrared light. One camera was positioned close (~0.5 m) to the setup to observe approaching females and one camera was farther away (~2 m) to observe bat attacks. Bat attacks were defined as an individual approaching the speaker and changing the course of its flight path within the video frame; a more detailed description of the criteria for approaching bats and female frogs has been published previously[60]. The speaker setup was placed away from any calling male or chorus. After the experiment, we counted all midges on the foil and measured noise and light levels at the playback site. Up to 20 individual midges were collected per trap and stored in alcohol for later identification (data not shown).

Mate choice tests. We recorded 22 males from 5 urban and 5 forest populations in a sound-attenuating box (30 cm by 32 cm by 34 cm (height by width by length)) with either an uncalibrated microphone (Sennheiser ME62) or calibrated microphone (G.R.A.S. 1/2 inch 46AE) protruding through the top of the box. Males were placed in recording boxes in a plastic container that was filled to the rim with 3 cm of water and stimulated to call using chorus sounds that were played back at low amplitude through a speaker (Ensemble IV, Cambridge SoundWorks). From each of these recordings, we selected a single call that had the greatest number of chucks and the highest amplitude. We constructed 10 different stimulus pairs containing an urban and forest male that was played in an alternating manner at 1 call every 2 s (thus keeping the call rates the same between call types). Call amplitudes were normalized in the program Audacity by setting the amplitude of the whine to −6 dB. The 20 stimuli ranged in call complexity from 0 to 3 chucks (average of 1.3 ± 1.37) and differed between urban and forest males by 0.8 chucks. For seven of the stimulus pairs, the urban call had more chucks than the forest call.

For a single pair, the urban call had one chuck and the forest call had no chucks. For another pair, the urban call had three chucks and the forest call had one chuck. The remaining five pairs had one versus two chucks, for forest and urban stimuli respectively. For three of the pairs for which we recorded calls with the calibrated microphone, we maintained the recorded amplitude differences (thus setting the amplitude of the loudest whine to −6 dB in the stereo file).

We tested females that were collected from urban or forest locations for their preference to approach the urban or forest male for each stimulus pair in a hemi-anechoic chamber under infrared lighting. Individual females were placed under a funnel in the centre of the room and were stimulated with a playback from two speakers on opposite sides that played either the urban or forest stimulus from a distance of 80 cm. The speakers were calibrated with a reference recording of an artificial whine set to 82 dB SPL (re. 20 μPa at 50 cm, measured with Extech Instruments SPL meter type 407764, set to C-weighted, fast and max). We raised the funnel after 2 min and scored the choice of the female (defined as staying 2 s within a 10-cm radius of the centre of a speaker) as well as approach latency (s). Starting order (first or second) and side (left or right) of the forest and urban call was randomized between trials. Experiments lasted until females made a choice. When females did not move for 5 min or did not make a choice within 10 min the experiment was abandoned.

Translocation experiments. We collected 112 male frogs from five different urban and five different forest populations and placed them in one of four different mesocosm environments. These mesocosms consisted of a pool (Intex Ocean, diameter of 183 cm) filled with soil, some plant material and two artificial puddles (diameter of 40 cm), covered with mosquito nets to exclude predators and parasites, and to keep the frogs in the pool. Chunks of conehead termite (*Nasutitermes corniger*) nests were added to each mesocosm to allow frogs to forage ad libitum. The mesocosms were placed in either a forested environment near Gamboa or an urban environment in Gamboa and contained 6–8 males from an urban or forest population (we never mixed populations). The mesocosm locations experienced overlapping noise levels and non-overlapping light levels between habitat types (49–51 dB SPL, 0.08–0.18 lx in the forest; 48–55 dB SPL, 0.96–1.21 lx in the urban environment). We repeated the experiment four times per treatment (resulting in 16 treatment trials on 16 different nights) and randomized and balanced the location of the mesocosms. We always collected males from the urban and forest populations on the same night. Male calling behaviour was recorded for 4 h on four consecutive nights on automatic sound recorders (SongMeter SM2, Wildlife Acoustics) starting at sunset.

Data analyses. We scored the call rate of each individual (number of calls per min) and the maximum and average complexity (number of chucks produced per call) in Audacity. Call rate and average complexity could not always be scored in a high-density chorus owing to many overlapping calls. For the field recordings, we selected a 1-min portion of continuous calling. Frogs in the translocation experiment did not always call on the first or second night in the mesocosms and we therefore analysed recordings from the third or fourth night. We randomly selected for each night up to eight 30-s portions either from the start of a calling bout or from a period with peak activity (determined based on the amplitude profile of the recording). We selected the portions from the first, second, third and fourth hour after sunset and analysed from each portion the calls of up to two different males. All measurements were averaged per individual. Videos from the field playback experiments were used to score the number of females approaching the speaker as well as the number of bats passing over or attacking the setup using previously described selection criteria[60]. Call rate and complexity do not covary with size in our study species[51] and preliminary analyses revealed that urban and forest sites did not differ in temperature (see above and Supplementary Table 3). We therefore did not adjust call parameters for size or temperature in our final analyses.

We analysed all our data on call behaviour, (a)biotic conditions and mate choice in R (v.3.2.2). We constructed GLMMs for each dependent variable using the package lme4 (v.1.13). For the field data models, we included paired population number as a random effect and habitat type (urban/forest) as a fixed effect. Models on average and maximum call complexity contained chorus size as an additional fixed effect. The model on male mass contained size (snout–vent length) as an additional fixed effect and vice versa. For maximum call complexity, we used a Poisson distribution with sqrt link function. For models of chorus size, call rate, vigilance distance, average call complexity, noise and light levels, and bat, midge and female attraction, we used a Gaussian distribution with an identity link function. Average call complexity, light and noise levels, canopy cover, and bat, midge and female attraction were rank-transformed to fit model assumptions. For the translocation experiment, we tested for both single and interaction effects of translocation environment (urban/forest mesocosm) and background environment (urban/forest male). We constructed GLMMs with a Gaussian distribution and identity link function and included time of day nested in experimental day, experimental round and male number as random effects. We used likelihood ratio tests to assess significant effects. We followed up on significant interaction effects by running a new model with the four treatment groups added as fixed effects and by constructing independent contrasts between the groups. Data on calling

behaviour recorded at the start or during peak activity of a call bout showed very similar patterns and we therefore only report results for the former. Data on the female choices were analysed with a binomial test for which we pooled choices for females collected from urban and forest environment as our sample sizes did not allow for testing of an interaction effect.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

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Author contributions

W.H. and J.E. conceived the study and designed the experiments. M.B., L.K., N.H. and S.G. collected the field data. M.B., L.K., S.G. and W.H. analysed the data. W.H., J.E., R.A.P., P.A.T., X.E.B. and M.J.R. discussed the results and wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

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