

Using mobile phones as acoustic sensors for high-throughput mosquito surveillance

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Abstract The direct monitoring of mosquito populations in field settings is a crucial input for shaping appropriate and timely control measures for mosquito-borne diseases. Here, we demonstrate that commercially available mobile phones are a powerful tool for acoustically mapping mosquito species distributions worldwide. We show that even low-cost mobile phones with very basic functionality are capable of sensitively acquiring acoustic data on species-specific mosquito wingbeat sounds, while simultaneously recording the time and location of the human-mosquito encounter. We survey a wide range of medically important mosquito species, to quantitatively demonstrate how acoustic recordings supported by spatio-temporal metadata enable rapid, non-invasive species identification. As proof-of-concept, we carry out field demonstrations where minimally-trained users map local mosquitoes using their personal phones. Thus, we establish a new paradigm for mosquito surveillance that takes advantage of the existing global mobile network infrastructure, to enable continuous and large-scale data acquisition in resource-constrained areas.

Introduction

Frequent, widespread, and high resolution surveillance of mosquitoes is essential to understanding their complex ecology and behaviour. An in-depth knowledge of human-mosquito interactions is a critical component in mitigating mosquito-borne diseases like malaria, dengue, and Zika (Macdonald, 1956; World Health Organization, 2014; Godfray, 2013; Besansky, 2015; Kindhauser et al., 2016). However, there is a paucity of high-resolution ecological data on the abundance, temporal variation, and spatial distribution of mosquito vector species. This poses a serious impediment to the effective control of mosquito-borne diseases (Townson et al., 2005; Ferguson et al., 2010; Hay et al., 2013). Efforts to map mosquito populations primarily rely on interpolative mathematical models based on factors such as clinical disease burdens or climatological data, with field inputs from entomological surveys being a comparatively sparse contribution (Hay et al., 2010; Mordecai et al., 2017). This scarcity of field data stems from the absence of high-throughput, low-cost surveillance techniques to map the distribution of mosquitoes. This effect is particularly severe in resource-constrained areas, as currently used methods like trapping and manual identification require considerable expense, labour, and time. However, for disease control strategies to be truly effective, it is critical for them to be strongly informed by current mosquito population distributions. Hence, there is a pressing need for novel methods of surveillance that can rapidly and inexpensively sample mosquito populations across large areas at high spatio-temporal resolutions.

41 Acoustic surveillance was proposed decades ago as a high-throughput automated surveillance
 42 approach, where mosquitoes in flight are identified using the species-specific frequencies in their
 43 wingbeat sounds (*Kahn et al., 1945; Kahn and Offenhauser Jr, 1949; Offenhauser and Kahn, 1949;*
 44 *Kahn and Offenhauser, 1949; Jones, 1964; Johnson and Ritchie, 2016*). This technique is based
 45 on the hypothesis that sexual selection has led to unique and characteristic sound signatures
 46 for different mosquito species (*Roth, 1948; Belton, 1994; Gibson and Russell, 2006; Cator et al.,*
 47 *2009; Warren et al., 2009; Pennetier et al., 2010; Cator et al., 2010*). However, the challenge of
 48 using expensive microphones to acquire low amplitude mosquito sounds against potentially high
 49 background noise poses a significant barrier to its widespread adoption as a field technique (*Jones,*
 50 *1964; Unwin and Ellington, 1979; Raman et al., 2007*). Optical wingbeat measurement has been
 51 proposed as a proxy for sound recording, to overcome the technological limitations in audio signal
 52 acquisition (*Moore et al., 1986; Batista et al., 2011; Chen et al., 2014; Silva et al., 2015*). However,
 53 this approach has not yet taken flight on a large scale, as the cost and global scalability of the
 54 requisite hardware remain challenges for its large-scale deployment.

55 Here, we propose a novel solution that uses mobile phones to enable widespread acoustic
 56 mosquito surveillance. We use the sensitive microphones in mobile handsets to record species-
 57 specific wingbeat sounds from a variety of mosquito vector species for automated identification
 58 and analysis (Figure 1A). Our technique exploits the rapid advances in mobile phone hardware
 59 technology, recognizing that these highly portable devices are optimized for sophisticated audio
 60 capture, processing, and transmission. Further, the proliferation of the global mobile network im-
 61 plies that around 5.3 billion users worldwide are connected today by a distributed data transmission
 62 infrastructure, growing quickly at over 6% annually (*Cerwall et al., 2017*). Mobile connectivity has
 63 revolutionized the delivery of services based on mapping and analyzing crowdsourced data from
 64 mobile phones, which has enabled many innovative applications in citizen science (*Graham et al.,*
 65 *2011; Catlin-Groves, 2012; Malykhina, 2013; Kong et al., 2016*). Also, the explosive growth in mobile
 66 phone use is most pronounced in Africa, Asia, and Latin America (*Cerwall et al., 2017*), which also
 67 bear the brunt of mosquito-borne diseases (*World Health Organization, 2014*). This overlap implies
 68 that a mobile phone based concept can be particularly scalable, sustainable, and cost effective for
 69 sampling real-time mosquito population dynamics in resource-constrained areas with high disease
 70 burdens. Our proof-of-concept study highlights the potential of our solution to engage citizen
 71 scientists around the world in mosquito surveillance, without the need for specialized equipment
 72 or extensive formal training.

73 Results

74 Mobile phone microphones can be used to record wingbeat sounds from mosquitoes

75 We record the acoustic signatures of free flying mosquitoes using mobile phones, by orienting the
 76 primary microphone towards the mosquito and using an audio recording application to acquire
 77 and store its wingbeat sound (Figure 1A,B, Figure 1 - figure supplements 1,2, Supplementary Audio
 78 SA1, Supplementary Videos SV1-3). Mosquito sounds have relatively low complexity. They comprise
 79 a single fundamental frequency with several overtones, which we identify using the short time
 80 Fourier transform (STFT) (Figure 1C). The sound from typical flight traces is not monotone, but shows
 81 natural frequency variations which we capture as a wingbeat frequency distribution, in a range
 82 characteristic of the given species (Figure 1D). Since mosquitoes rarely fly at speeds over half a meter
 83 per second, the Doppler shift of frequency during free-flight is small ($1 - [330 - 0.5/330 + 0.5] \approx 0.3\%$,
 84 i.e. < 2 Hz) when compared to the observed natural spreads of up to 100 Hz within a single flight
 85 trace. Sexual dimorphism in most species implies that males have higher frequencies than females.
 86 Female wingbeat frequencies are typically between 200 to 700 Hz. This overlaps the voice frequency
 87 band (300 to 3000 Hz), in which phones are designed to have maximum sensitivity. In addition,
 88 using mobile phones as recording platforms automatically registers relevant metadata parameters,
 89 such as the location and time of data acquisition. This adds valuable secondary information to

90 acoustic data that is useful for species identification and spatio-temporal mapping. Such acoustic
 91 and spatio-temporal information could potentially be aggregated from many mobile phone users,
 92 to generate large data sets that map the distribution of mosquito species at high resolutions (Figure
 93 1 - figure supplement 1).

94 **Mobile phone microphones accurately capture wingbeat frequencies without dis-** 95 **tortion**

96 Our concept of mobile phone based acoustic surveillance is based on the idea that mobile phones
 97 are high fidelity acoustic sensors, which faithfully capture the frequencies produced by mosquito
 98 wingbeats during flight. To confirm this, we compared measurements of wingbeat frequency from
 99 two independent modalities, high speed video and mobile phone audio (Sony Xperia Z3 Compact),
 100 for female *Culex tarsalis* mosquitoes in tethered flight (Figure 2A, Supplementary Video SV4). For
 101 synchronized audio-video recordings, spectrograms were aligned in time to within 2 ms, and show
 102 an exact match in frequency at each time window to within a computational error margin of 2 Hz
 103 (Figure 2B). The respective distributions of wingbeat frequency have maximum sample fractions
 104 occurring in the same frequency bin (Figure 2C), giving the same median frequency of 262 Hz,
 105 despite the audio signal being noisier than the video. To show similarity between distributions, we
 106 compute the Bhattacharya Coefficient (BC) - a measure of the mutual overlap of two distributions,
 107 ranging between 1 for completely coincident and 0 for completely separated distributions. We
 108 obtain a high BC of 0.95, implying an almost complete overlap between the distributions. This
 109 corroborates the spectral accuracy of wingbeat frequency distributions recorded by mobile phones,
 110 based on an independent optical reference.

111 **Mobile phone microphones are comparable to studio microphones in recording** 112 **mosquito wingbeats at up to 100 mm**

113 We propose using mobile phones for acoustic surveillance under field conditions, where the pri-
 114 mary challenge is recording faint mosquito sounds in noise-prone environments. Accordingly, it
 115 is important to establish the limits within which their built-in microphones are sensitive enough
 116 to reliably acquire data with high signal-to-noise ratios (SNR). The technical specifications of many
 117 commercial mobile phone microphones are not openly available. Hence, we experimentally cali-
 118 brated a range of mobile phone models against two reference electret condenser microphones
 119 under identical conditions. Using a piezoelectric buzzer of constant amplitude (77 dB at source)
 120 and frequency (500 Hz) as a standardized sound source, we first showed that both smartphones
 121 (iPhone 4S, Xperia Z3 Compact) and low-end feature phones (SGH T-209 clamshell model) have
 122 SNRs comparable to the reference microphones over distances up to 100 mm (Figure 2D). Next,
 123 we recorded wingbeat sound from tethered mosquitoes to gauge suitable working distances over
 124 which SNR is high enough to allow detection. Curves of mobile phone SNR over distance indicate
 125 that all the phones tested, including a decade-old basic phone (SGH T-209), are capable of acquiring
 126 detectable wingbeat sound up to 50 mm from a mosquito (Figure 2E). This is a working distance we
 127 have found to be easily achievable when making free-flight recordings in the field. This distance
 128 improves to 100 mm in the case of smartphones like the Xperia, making it even easier for users to
 129 record mosquitoes (Figure 2 - figure supplement 1). Beyond these distances, the mosquito sound
 130 becomes imperceptible, drowned out by background noise. We also caution that these working
 131 limits are appropriate in quiet to moderately noisy environments with background noise levels be-
 132 low 50 dB, and shrink drastically in louder conditions. In addition to distance, the orientation of the
 133 mobile phone is also critical to maximizing SNR, as its acoustic sensitivity is directional and localized
 134 around the primary microphone. Thus, the success of using mobile phones to record mosquitoes
 135 is fundamentally dependent on active involvement from the user, to maintain the appropriate
 136 distance and orientation of the phone with respect to the mosquito, and avoid introducing noise
 137 via bumps and scrapes of the microphone.

138 **Diverse mobile phone models acquire quantitatively equivalent acoustic data from**
 139 **mosquitoes**

140 There are over a 100 mobile phone brands in use worldwide, with a range of devices widely varying
 141 in features and capability. Widespread mobile phone based surveillance is only possible if mosquito
 142 wingbeat sound collected using any phone can be analyzed using the same identification metrics
 143 and algorithms. This means that a diverse range of phone models must record quantitatively
 144 equivalent acoustic data with identical spectral profiles. This is a necessity for our proposed
 145 surveillance technique to scale to the broadest possible user base, particularly in countries with
 146 lower smartphone penetration. We compared eight different smart and feature phones ranging
 147 in price between ~ \$20 and ~ \$700 (Figure 2F), by analyzing 5 minutes of audio recorded with
 148 each phone from free-flying female *Anopheles stephensi* mosquitoes of similar age from a lab-
 149 reared population contained in a cage. We noted high degrees of overlap in wingbeat frequency
 150 distributions recorded by each phone for this population, with both mean and median frequencies
 151 obtained by each phone differing by less than 5% from other phones (Figure 2F).

152 We computed the Bhattacharya Coefficient (BC) and obtained high degrees of overlap between
 153 0.93 to 1 (Figure 2H). We also quantified how distinguishable these wingbeat frequency distributions
 154 are, using the Jensen-Shannon divergence metric (JSD) between each pair of phones (Figure 2G).
 155 This metric, which ranges from 0 for identical to 1 for completely disparate distributions, can be
 156 inversely related to the amount of mutual information shared between the distributions. The
 157 JSD for each pair of phones had low values below 0.3 indicating high similarity in measurement,
 158 corroborating that wingbeat frequency sampling is relatively insensitive to the phone used. This is
 159 also true of other species we surveyed, with the JSD between phones typically between 0.1 to 0.35
 160 for a given population. This range of JSD provides an estimate of the variance inherent in sampling
 161 a population of the same species. Hence, the upper end of this range gives an approximate
 162 quantitative threshold for differentiability requirements between wingbeat frequency distributions
 163 of different species. Thus, we demonstrate that a diverse range of both smart and feature phones
 164 provide highly similar acoustic spectra from the same population of mosquitoes, enabling a truly
 165 universal acoustics-based platform for mosquito identification.

166 **Mosquito species have characteristic wingbeat frequency distributions that are**
 167 **measurable using mobile phone recordings**

168 The most important ingredient for acoustic identification of mosquitoes is prior knowledge of the
 169 wingbeat frequency distributions corresponding to different species. We surveyed representative
 170 populations from 20 medically relevant mosquito species under similar experimental conditions
 171 (Figure 3A), to establish a reference dataset for classifying acoustic recordings (*Mukundarajan et al.,*
 172 *2017*). Although we recorded mosquitoes using a variety of phones, the results presented here
 173 focus on data acquired by the 2006-model \$20 SGH T-209 feature phone. This highlights that even a
 174 basic low-end phone is capable of collecting high quality acoustic recordings, to precisely measure
 175 wingbeat frequency distributions.

176 We analyzed the recordings of the reference populations by dividing their spectrograms into 20
 177 ms sample windows, and applying a peak finding algorithm in each window to measure instanta-
 178 neous frequency. The number of frequency values we obtained for each species had a wide range
 179 between 300 to 50,000 data points, and these were binned into wingbeat frequency distributions
 180 (Figure 3A). To verify whether these reference distributions are sufficiently well-sampled, we drew
 181 random subsets of frequency values from each distribution, and measured the statistical distance
 182 from these subsets to their parent distributions. For subsets of increasing size, we observed a
 183 rapid convergence of the BC and JSD to their respective values of 1 and 0 expected for distributions
 184 identical to the parent. We showed computationally that that the length of the smallest subset of
 185 randomly selected values has a BC above 0.9 compared to the full distribution is a small fraction
 186 (less than 0.2) of the total number of samples in the full distribution for each species. Some species,

187 such as *An. dirus* and *An. stephensi*, show slower convergence, due to the smaller sample sizes used
 188 to build the frequency distributions. The time for convergence above a BC of 0.9 corresponds to
 189 approximately 60 samples, or 1.2 seconds of recording time, to generate a well-sampled wingbeat
 190 frequency distribution for a given population. Since our reference distributions are recorded for
 191 between 6 to 1000 seconds each, we conclude that they are sufficiently representative of the varia-
 192 tions within the reference population. Nevertheless, it is also clear that the longer a population is
 193 sampled for, the better the frequency distribution constructed to represent it.

194 We also considered whether significant variations exist between different colonies or popula-
 195 tions within a species. We found that different lab-reared strains of *Anopheles gambiae*, including
 196 permethrin susceptible and resistant variants sourced from Kenya and an additional bendiocarb
 197 resistant strain sourced from Benin, produced highly similar frequency distributions with large
 198 overlaps of 0.84 to 0.89 as measured by their mutual BCs (Figure 3 - figure supplement 1C). Similarly
 199 for *Aedes aegypti*, lab-reared mosquitoes from two separate colonies of the same New Orleans
 200 origin, as well as first generation progeny from eggs retrieved from the field in Los Angeles, had
 201 high overlaps in their frequency distributions with mutual BCs ranging between 0.66 and 0.74 (the
 202 slightly lower BC values arise due to the distribution for one of the colonies having a smaller spread
 203 despite being completely overlapped by the other two) (Figure 3 - figure supplement 1C). Thus, we
 204 hypothesize that it may be possible for certain species to have wingbeat frequency distributions
 205 that are universal across colonies and strains, facilitating automated identification using the same
 206 reference. However, we also find that lab-reared strains of *Anopheles arabiensis* originally sourced
 207 from Senegal and Sudan have non-overlapping frequency distributions, with a very small BC of
 208 0.14 (Figure 3 - figure supplement 1C). This signals that there may be other factors underlying
 209 geographic variability in wingbeat frequency within a species, which merit further investigation.
 210 Hence, it is advisable to build local acoustic databases wherever possible to improve classification,
 211 by recording actual frequency distributions from mosquitoes whose species have been identified
 212 by non-acoustic methods (Figure 1 - figure supplement 1A-D).

213 **Estimation methods can be used for species classification of acoustic recordings**

214 The core concept of acoustic surveillance is the correct identification of the species of a mosquito
 215 given its wingbeat acoustic signature. Although previous approaches have used a single averaged
 216 value of frequency to represent a flight trace (*Moore et al., 1986; Batista et al., 2011; Chen et al.,*
 217 *2014; Silva et al., 2015; Villarreal et al., 2017*), our approach takes into account the natural variance
 218 within each trace, by treating the collection of frequency values across all time windows of the
 219 recording as a distribution. We classify a given flight trace by statistically comparing this distribution
 220 against the known wingbeat frequency distributions for different species. Although classification is
 221 possible through several approaches that emphasize different statistical comparisons, in this work
 222 we apply the method of Maximum Likelihood Estimation (MLE). Here, the species is considered to
 223 be a parameter, whose discrete values affect the statistical distribution of wingbeat frequency over
 224 a specified range. We classify a given flight trace as that species whose corresponding wingbeat
 225 frequency distribution is most likely to have produced the observed frequency values from that
 226 trace, with a confidence level equal to the likelihood estimate. The reference distributions for
 227 comparison are built using the data presented in Figure 3A, collected using the SGH T-209 basic
 228 phone.

229 The best indicator for how likely it is for recordings from a given species to be correctly classified
 230 is the degree of overlap of its characteristic wingbeat frequency distribution with other potentially
 231 confounding species. One way to measure this is the mutual JSD between pairs of species, which
 232 must be significantly higher than the threshold of 0.35 established earlier for the variance inherent
 233 within the same species. The vast majority of all possible pairwise combinations of species in
 234 our study (184 out of 190) had JSD greater than this minimum value (Figure 3B), indicating that
 235 differences in frequency distribution between species are significant in most cases (Figure 3C). We
 236 translate this into probabilistic estimates of classification accuracy, based on the principle that

237 randomly drawn samples from a given species' frequency distribution will be classified with an error
 238 rate that reflects its overlap with other species' distributions. We applied the MLE algorithm on
 239 bootstrapped subsets from the wingbeat frequency data used to create the reference distributions,
 240 to build a confusion matrix showing the relative proportions in which subsets derived from different
 241 species are classified correctly or erroneously. Our results verify that species with low overlap in
 242 wingbeat frequency are almost always correctly classified, while those with high degrees of overlap
 243 are most likely to be mutually misclassified. This provides quantitative estimates of the inherent
 244 degree of differentiability of each species with respect to all others (Figure 3D), setting intrinsic
 245 limits on the success of any classification scheme based exclusively on wingbeat frequency.

246 The classification accuracies and error rates for samples bootstrapped from the same data
 247 used to build reference distributions represent the best case scenario. A more realistic test of
 248 our classification approach is its ability to handle new flight traces that are not part of the original
 249 reference datasets. We validated the MLE algorithm by classifying recordings from the iPhone
 250 4S, Sony Xperia Z3 Compact, and Google Nexus One (Figure 3E, Figure 3 - figure supplement 1D),
 251 by comparing them to the reference dataset collected using the SGH T-209 (Figure 3A). This is a
 252 validation dataset that has similar species frequency distributions as the reference dataset, but also
 253 incorporates the additional variance arising from using different phones. We sequentially tested a
 254 large number of individual flight traces chosen at random from eight different species, to simulate
 255 the stochastic nature of human-mosquito encounters involving a variety of species in the field.
 256 Each flight trace was compared against all species in the reference dataset, and the best match
 257 was chosen. We observe that classification accuracies are slightly lower than those obtained for
 258 bootstrapped reference data (Figure 3E, Figure 3 - figure supplement 1D), indicating that our MLE
 259 approach is inherently limited in practice by overlaps in wingbeat frequency distributions.

260 **Acoustic data can be combined with metadata to improve classification accuracies**

261 The limitations of using wingbeat frequency as the sole distinguishing characteristic between
 262 species are exposed by the inability to perfectly classify species that have overlapping frequency
 263 distributions. A typical wingbeat frequency distribution for any given species has a spread of
 264 150 to 200 Hz between the 5th to 95th percentiles, located within the frequency range of 200
 265 to 700 Hz for most female mosquitoes. But with over 40 species of medical relevance among
 266 *Anopheles* mosquitoes alone, the pigeon-hole principle makes it impossible to have perfectly non-
 267 overlapping distributions for all species (Chen *et al.*, 2014). Unlike speech (Rabiner and Schafer,
 268 2011), music (Wang *et al.*, 2003), or other insects (Drosopoulos and Claridge, 2005), birds (Marler
 269 and Slabbekoorn, 2004) and animals (Mellinger *et al.*, 2007; Mellinger and Clark, 2000; Portfors,
 270 2007), the low time-domain complexity of mosquito sound makes it difficult to identify temporal
 271 motifs or other acoustic parameters that can be used in species identification (Chen *et al.*, 2014;
 272 Kiskin *et al.*, 2017). To improve the classification of mosquito species, Chen *et al.* (Chen *et al.*,
 273 2014) introduced the concept of metadata, where parameters like the time or place of recording
 274 can be used as additional features to differentiate between mosquitoes with varying circadian
 275 activity or geographic distribution. Mobile phones offer the advantage of automatically registering
 276 time stamps for acoustic data, and location tags from cell tower or GPS measurements, along with
 277 sensors to collect other metadata to support identification such as photographs. We assessed the
 278 effect of including even a single metadata parameter to refine species classification, by applying
 279 a location filter based on entomological survey data from over 200 countries for the 20 species in
 280 our database (Walter Reed Biosystematics Unit, 2017). This data is available in the public domain
 281 (Walter Reed Biosystematics Unit, 2017), and can be readily incorporated as prior knowledge to
 282 aid in classification. We repeated our sequential classification of flight traces from randomly chosen
 283 species, with each recording randomly assigned to one of the countries where that species is
 284 known to be found. Recordings are then only compared among the subset of species co-located
 285 in that country. This significantly improved classification accuracy not only for individual species,
 286 but also from about 35% to 65%, as an average over all the species tested (Figure 3E, Figure 3 -

287 figure supplement 1D). In earlier work, algorithms for mosquito species classification have achieved
 288 accuracies of around 80%, when comparing a smaller number (10) of insect types. This included both
 289 sexes of four acoustically dimorphic mosquito species, and two species of flies (*Chen et al., 2014*).
 290 Although the overall classification accuracy by our method is a little lower at 65%, we note that this is
 291 for twice the number of species and includes only female mosquitoes. Since classification accuracy
 292 decreases significantly with the number of species considered and their degree of frequency overlap,
 293 the accuracies produced by our classification algorithm appear to be reasonable in comparison
 294 to earlier work. We underline the caveat that in practice, classification accuracies can be further
 295 lowered by species other than the ones in our reference database. However, the inclusion of a
 296 location filter eliminates potential errors from irrelevant species, and highlights the discriminatory
 297 power of such metadata.

298 For greater clarity, we illustrate a few scenarios where acoustic data can be combined with
 299 metadata for identifying mosquitoes in field investigations (Figure 3 - figure supplement 2). In the
 300 simplest case, species with completely non-overlapping frequency distributions, such as *Anopheles*
 301 *gambiae* and *Culex pipiens* ($JSD = 1$, negligible probability of mutual mis-classification), can easily
 302 be distinguished by sound alone (Figure 3 - figure supplement 2A). Some species with overlapping
 303 frequency distributions can still be perfectly classified using location metadata on the basis of
 304 their spatial segregation. For instance, *An. atroparvus* has a 38% probability of being mis-classified
 305 as *An. dirus* (overlapping frequency distribution with mutual $JSD = 0.26 < 0.35$). However, this
 306 confusion never occurs when location is taken into account, as these species are respectively
 307 found in Europe and South Asia (*Sinka et al., 2012*), and do not co-exist in the same area (Figure 3 -
 308 figure supplement 2B). The use of timestamps has been suggested previously in the case of species
 309 which are overlapping in both frequency and spatial distribution, but have different circadian activity
 310 (*Chen et al., 2014; Silva et al., 2015*). As an example, *Ae. aegypti* is often misclassified as *An. gambiae*
 311 with high probability based on wingbeat frequency (mutual $JSD = 0.37 \sim 0.35$, *Ae. aegypti* wrongly
 312 classified 95% of the time). However, classification accuracy may possibly be substantially improved
 313 if recordings collected during daytime were all classified as *Ae. aegypti*, since it is much more likely to
 314 be active during daylight hours (Figure 3 - figure supplement 2C). Co-occurring and morphologically
 315 similar species of interest that can be acoustically distinguished include the arboviral vectors
 316 *Ae. aegypti* and *Ae. albopictus* (*Brogdon, 1994*) ($JSD = 0.55 > 0.35$, correctly distinguished by MLE
 317 around 98% of the time), and the closely related species *Cx. pipiens* and *Cx. quinquefasciatus*
 318 ($JSD = 0.65 > 0.35$, correctly distinguished around 95% of the time) (Figure 3 - figure supplement
 319 2D). Morphologically indistinguishable vector species like members of the *Anopheles gambiae s.l.*
 320 complex are of particular interest for acoustic identification (*Brogdon, 1998; Wekesa et al., 1998;*
 321 *Tripet et al., 2004*). Our results for four members of this complex imply significant distinguishability
 322 for *An. arabiensis*, *An. quadriannulatus*, *An. gambiae*, and *An. merus*. The JSD for all but one
 323 species pair ranges between 0.61 and 0.91 (Figure 3 - figure supplement 2E), and *An. gambiae* and *A.*
 324 *quadriannulatus* are correctly identified with over 97% probability. However, the pair of *An. arabiensis*
 325 and *An. merus* has high frequency overlap ($JSD = 0.29 < 0.35$, *An. arabiensis* is misclassified as *An.*
 326 *merus* with around 15% probability) (Figure 3 - figure supplement 2F). Improving classification rates
 327 in this case may not be possible without additional knowledge of their specific local ecology, such as
 328 the distribution of saltwater breeding sites for the halophilic *An. merus*. Despite such limiting cases,
 329 the wingbeat frequency combined with phone provided metadata has significant discriminatory
 330 power between species in many situations, making mobile phone based acoustic surveillance an
 331 extremely promising screening tool for mosquito population compositions (Figure 3C).

332 **Mobile phones can be effectively used to map mosquito activity through user-** 333 **driven acoustic surveillance**

334 So far, we have calibrated the performance of mobile phones and studied acoustic frequency
 335 dispersion among different mosquito species, using lab-reared populations in controlled environ-
 336 ments. We now test our mobile phone based surveillance approach under field conditions, by

337 collecting mosquito wingbeat data in various settings and measuring acoustic variance among
338 wild mosquito populations. Using the techniques described earlier (Figure 1 - figure supplement
339 2, Supplementary Videos SV1-3), we recorded mosquitoes not only in free flight, but also under
340 confinement in cups or bottles to amplify the sound and increase the length of flight traces, due to
341 the enforced proximity to the microphone. We also collected recordings in a wide range of ambient
342 environments, from urban and rural homes, both indoors and outdoors, to forests and parks (Figure
343 4 - figure supplement 1, Supplementary Audio SA2-7). We obtained mosquito recordings with high
344 SNR, whose distinctive narrow-spectrum characteristics allow their easy visual identification in
345 spectrograms against background noises such as birdsong, fire truck sirens, and human speech. We
346 matched the sound signatures against our frequency distribution database (Figure 3A for females,
347 data for males not shown) to identify their species, which we also confirmed by capturing the
348 respective specimens for morphological identification by microscopy.

349 Wild mosquitoes in field environments may vary considerably in terms of age, body size, rearing
350 temperature, and nutrition status, yet our method requires the variation in their wingbeat frequen-
351 cies to be sufficiently small in order to be identifiable. We explored the variations in wingbeat
352 frequency for flight traces corresponding to over 80 individual free-flying *Aedes sierrensis* mosquitoes,
353 recorded by volunteers at Big Basin Redwoods State Park in California, USA (BBR) (Figure 4 - figure
354 supplement 2A). Wingbeat frequency distributions for a population typically span a characteristic
355 frequency range with a spread of around 150 to 200 Hz (Figure 3A). For individual flight traces
356 collected in the field, the distributions lie at different positions within the population-level frequency
357 range, and have spreads (difference between 5th and 95th percentile) between 10 to 100 Hz (Figure
358 4 - figure supplement 2B,C). The spread in frequency for a single flight trace is typically smaller than
359 the spread across a population, with the difference between mean frequencies for different traces
360 contributing to the additional variation in the population's frequency distribution. This spread
361 in each flight trace also appears uncorrelated to the duration of the trace itself (Figure 4 - figure
362 supplement 2B,C), suggesting that it may arise due to aerial maneuvers during flight. Although the
363 mean frequency for most individuals is tightly clustered, there are still outliers that pose challenges
364 for classification (Figure 4 - figure supplement 2B). Additionally, the classification accuracy for any
365 given recording was not a function of the duration of the trace but of the mean frequency, indicating
366 that overlaps between frequency distribution play the fundamental role in identifying the species
367 correctly, no matter how long the flight trace is recorded for (Figure 4 - figure supplement 2D).
368 We also observed that frequency distributions for individual flight traces have a minimum spread
369 of around 2% of the mean frequency irrespective of the mean frequency or the duration of the
370 recording, suggesting that this is an inherent natural variation in the wingbeat frequency during free
371 flight (Figure 4 - figure supplement 2C). Further, both the spread and the duration of the recording
372 did not affect whether the recording was classified correctly (Figure 4 - figure supplement 2E),
373 corroborating the importance of wingbeat frequency as the primary identifying factor.

374 Wingbeat frequency for insects, including mosquitoes, has been shown to vary linearly with
375 temperature (Oertli, 1989; Villarreal et al., 2017). It may be possible to correct for this factor by
376 measuring ambient temperature when recording as part of relevant metadata, as suggested in other
377 studies (Chen et al., 2014). We also investigated correlations between wingbeat frequency and body
378 size, by comparing the physical specimens corresponding to several flight traces (Figure 3F,G). For
379 mosquitoes varying almost two-fold in size (Figure 3G), the difference in mean frequency between
380 each mosquito is less than the spread within each individual flight trace, and non-monotonic with
381 body size (Figure 3F). This provides a field confirmation of lab studies that show no relationship
382 between wingbeat frequency and body size (Villarreal et al., 2017). Despite the variations among
383 individuals, our MLE approach (using a pool of all field recordings together to create a reference
384 frequency distribution) was able to correctly classify recordings from wild *Aedes sierrensis* mosquitoes
385 in about two-thirds of cases (54 out of 82 recordings), when considering other local species (*Cx.*
386 *pipiens*, *Cx. quinquefasciatus*, *Cx. tarsalis*, and *Cu. incidens*) as potential confounders.

387 We next show how mobile phone recordings of mosquitoes in the field can be pooled from

388 minimally trained users for spatio-temporal mapping, using the time and location metadata associ-
389 ated with each recording. Our recordings of wild *Ae. sierrensis* mosquitoes in BBR (Figure 4 - figure
390 supplement 2A) represent the only species found in the park, based on microscopic identification of
391 the corresponding captured specimens. These were collected over a continuous 3 hour period by 15
392 volunteers hiking along trails and recording with their personal mobile phones. The recordings were
393 mapped according to their locations to reflect spatial variations in mosquito distribution within the
394 park (Figure 4D). Parsing this data by recording time further reveals the rise and fall in the number
395 of human-mosquito encounters over an evening (Figure 4D inset), which can be correlated to the
396 circadian activity of *Ae. sierrensis*.

397 In addition to the temperate forest environment represented by BBR, we carried out another
398 small-scale field trial in a tropical village, at Ranomafana (District Ifanadiana) in Madagascar (RNM).
399 Here, our first step was to establish the baseline species composition, by collecting live mosquitoes
400 in traps or bags and identifying them using microscopy. We found both *Anopheles* and *Culex*
401 mosquitoes, which we identified only to genus level in the field. We recorded several minutes of
402 acoustic data from both, to form a frequency distribution reference database specific to the RNM
403 field site, for comparing new data from phone recordings (Figure 4A,B). We obtained two clearly
404 separated frequency distributions with a single peak each, implying that the two genera were
405 easily distinguishable by wingbeat frequency alone. About 60 recordings were collected in a 3-hour
406 period by 10 volunteers who were trained for about 15 minutes and stationed at specific locations
407 in the village. This number of recordings exceeded the number of mosquitoes simultaneously
408 captured in CDC light traps placed at the same locations. Mapping the distributions of the two
409 genera of mosquitoes across the village revealed considerable heterogeneity in the proportions
410 of *Anopheles* and *Culex* mosquitoes from the western riverside to the eastern hillside, possibly
411 influenced by extremely local factors such as drainage, density of humans, or presence of livestock
412 (Figure 4C). This heterogeneity was concordant with the data from CDC light traps, which collected
413 *Anopheles* mosquitoes only in those locations where they were also recorded by volunteers (Figure
414 4C, insets). Although the ratio of the two kinds of mosquitoes varied between acoustic recording
415 and conventional trapping at each location, there was a qualitative correspondence between the
416 two methods in terms of the relative number of mosquitoes at a location, and the more numerous
417 species.

418 Together, the two pilot field trials at BBR and RNM highlight spatial variations in mosquito
419 distributions along with their circadian activity patterns, in very different field environments. The
420 greater number of observations obtained by pooling mobile phone recordings indicates that this
421 may be a more productive means of sampling mosquito populations as compared to trapping. At
422 the same time, the maps of mosquito activity at both field sites were extremely localized, on the
423 level of minutes and tens of metres. This demonstrates how mobile phone based crowdsourcing can
424 simultaneously enhance both the scale and the resolution of ecological measurements of mosquito
425 populations, indicating considerable potential for such an approach to the spatiotemporal mapping
426 of mosquito vectors.

427 Discussion

428 In this work, we demonstrate a new technique for mosquito surveillance, using mobile phones as
429 networked acoustic sensors to collect wingbeat frequency data for automated identification. We
430 present quantitative analyses of mobile phone acoustic signal quality and the differentiability of
431 important mosquito species, complemented by field data collected by minimally trained volunteers
432 and organized into spatio-temporal maps.

433 The involvement of local volunteers in our study underlines that practically anyone with a mobile
434 phone can quickly be trained to contribute data towards mosquito surveillance efforts. Based on
435 the proof-of-concept presented here for a mobile phone based surveillance framework, we propose
436 a citizen science effort for mapping mosquito populations (Figure 1 - figure supplements 1,2). Citizen
437 science is an exciting paradigm for community-based vector surveillance, with emerging initiatives

438 such as the United Nations-backed Global Mosquito Alert. These programs rely on data collected by
439 volunteers from the general public, in the form of physical specimens or through apps for reporting
440 the presence of mosquitoes with photographs for expert identification. Our proposed platform
441 would support the collection of mosquito sound recordings from mobile phone users, automating
442 the audio processing to instantly identify and map the species without human intervention. This
443 approach has the potential to greatly expand surveillance efforts in resource-limited areas where
444 they are needed the most, by enabling people to take the initiative in tracking disease vectors within
445 their own communities.

446 The greatly reduced cost of surveillance as a result of using pre-existing infrastructure, together
447 with the ability to localize mosquito recordings, allows both the coverage and resolution of mosquito
448 field maps to increase. The availability of sufficient data on highly local scales could inform mosquito
449 control strategies tailored to a given location. Further, this approach also has the potential to screen
450 for the presence of exotic or invasive species, particularly in high-risk areas such as port cities.
451 Additionally, it provides a rapid and easy way to survey baseline mosquito populations in a given
452 area and their fluctuations over time, which may be highly relevant for programs that aim to control
453 mosquito populations through the release of sterile or genetically modified organisms. The implicit
454 need for human involvement in our method skews the collection strongly towards species that
455 are most likely to encounter a human. Although all methods of mosquito collection, including
456 various traps, have biases in the species they predominantly attract, the over-representation of
457 anthropophilic species is a caveat to be noted when probing mosquito ecology using our method.

458 We have focused on statistical Maximum Likelihood Estimation methods for acoustic identifica-
459 tion of mosquitoes using known distributions corresponding to different species as a reference.
460 There are however many possible methods to predict the species that a given recording belongs to,
461 with various techniques for incorporating prior knowledge about the wingbeat frequency, circadian
462 activity, habitat and other characteristics of the relevant mosquito species. The field of computa-
463 tional entomology, which includes the development of algorithms for the automated identification
464 of mosquito sound in acoustic data and its classification by species is an active area of research, with
465 growing relevance for the automation of mosquito surveillance (*Chen et al., 2014; Silva et al., 2015;*
466 *Kiskin et al., 2017*). There is considerable scope for studies that not only search for more acoustic
467 features that discriminate between mosquito species, but also those which use spatiotemporal
468 population data to identify trends and patterns. These can be correlated to other man-made and
469 environmental factors, to enable evidence-based decision making for mosquito control and disease
470 management. Such insights require not only the development of algorithms capable of sifting
471 through surveillance data, but also considerable expansion of our databases of prior knowledge to
472 train these algorithms to identify more species across diverse areas of the world.

473 Recent technological trends have spurred a rapid rise in apps and services based on crowd-
474 sourcing data about the physical world through embedded sensors and user input from mobile
475 phones. Applying analytics methods and spatiotemporal mapping on this data provides useful
476 information ranging from traffic density to restaurant recommendations to earthquake warnings.
477 At present, the critical missing link in enabling similar advances in mosquito surveillance and control
478 is the capacity to generate large quantities of mosquito ecological data on fine-grained space and
479 time scales (*Ferguson et al., 2010*). Our mobile phone based solution holds great promise as a
480 scalable, non-invasive, high-throughput and low-cost strategy to generate such data, by leveraging
481 widely available hardware and an existing network infrastructure. We hope that a citizen science
482 approach to mosquito surveillance based on this method will boost our capability to dynamically
483 assess mosquito populations, study their connections to human and environmental factors, and
484 develop highly localized strategies for pre-emptive mosquito control (*Hay et al., 2013*).

485 **Methods and Materials**

486 **Acquisition of acoustic data from mobile phones**

487 Different mobile phones were used to collect sounds from mosquitoes, using audio recording
488 software that was already freely available on the devices, including applications for voice messaging,
489 voice memos, or sound recording. To locate the primary microphone on the body of the mobile
490 phone, we either read the location off a user manual showing its different components, or found the
491 microphone by trial-and-error, where we tapped the phone periphery and observed the response of
492 the recording software to locate the area with maximum audio sensitivity. The primary microphone
493 is oriented towards the mosquito for maximum sensitivity in audio acquisition. Audio data from the
494 phones was compiled and transferred to a server for processing. This method applies to all figures.

495 **Processing of acoustic data from mobile phones**

496 The audio signals from mosquitoes were acquired at different sampling rates ranging from 8 kHz
497 to 44.1 kHz and a variety of file formats, depending on the mobile phones and the specific in-built
498 or user-defined settings on the recording applications used to acquire the signal. When a raw
499 signal was acquired from the phone, it was converted into the WAV format for convenience of
500 processing, with sampling rate interpolated to 44.1 kHz if sampled at a lower rate. To reduce constant
501 background noise in the signal, we used a spectral subtraction algorithm, with the fundamental
502 principle of subtracting the actual or expected frequency content of pure noise from the spectrum
503 of the noisy signal. Here, we identified the background using an automated algorithm as those
504 spectral bands that are constant with almost zero variation in amplitude and frequency across
505 the entire sound clip. To generate the audio frequency spectrum over different instants of time,
506 we applied the Short Time Fourier Transform (STFT ; Signal Processing Toolbox, MATLAB R2015B)
507 to produce a spectrogram with resolutions of 5 Hz in frequency and 20 ms in time, having a high
508 degree (90%) of overlap between windows to achieve a trade-off between sufficient frequency
509 resolution and accurate localization of the signal in time.

510 **Construction of wingbeat frequency distributions (*Mukundarajan et al., 2017*)**

511 Using the spectrogram, we construct histograms for the distribution of peak frequencies for
512 mosquitoes of a given species. Once a sound was identified (either manually or using an automated
513 code) as belonging to a mosquito of a given species, the lowest frequency corresponding to a local
514 maximum in amplitude was detecting using a peak finding algorithm. In cases where more than
515 one mosquito is recorded sequentially in the same file, they are considered separate instances,
516 distinguished as contiguous durations of peak frequency in a given range separated by time. In
517 cases where recordings from mosquitoes overlap, the louder mosquito is considered. In many
518 studies, a representative wingbeat frequency is found for a single flight trace by averaging the
519 fundamental frequency over all time instants. However, the wingbeat frequency from a single
520 continuous mosquito signal can vary over a range of about 100 Hz for a tethered mosquito, and over
521 a few tens of Hz for mosquitoes in free-flight. To take into account this natural variation in wingbeat
522 frequency over the duration for an audio signal, we did not average the frequency over time, but
523 instead focused on the instantaneous frequency computed in each 20 ms interval time window.
524 Each time window was treated as an individual instance, and its fundamental frequency added
525 to a histogram, to bring out the natural variations in wingbeat frequency within a single signal.
526 Peak frequencies from all time windows are binned with a bin size of 5 Hz - the same frequency
527 resolution imposed upon the spectrogram. This yields a histogram that captures the distribution
528 of peak frequencies for that mosquito species, without making *a priori* assumptions about the
529 nature of this distribution. For statistical computations, the histograms are normalized by the total
530 number of instances such that the area under the distribution sums up to 1, yielding a discrete
531 probability density distribution for wingbeat frequency. However, for ease of representation in
532 figures, probability mass functions where the histogram counts are normalized relative to the

533 maximum number of counts in a single bin are shown, so that each histogram spans 0 to 1 on the
 534 y-axis. This method applies to all figures.

535 **Statistical tests and metrics**

536 Wingbeat frequency was represented as discrete probability density distributions, with the fre-
 537 quency binned into intervals of 5 Hz (the computational accuracy for frequency in our STFT analyses)
 538 and the area under the probability density distribution summing up to 1.

539 The peak frequency measured in each time window of the video or audio was treated as an individ-
 540 ual sample, and the 2-sample T-test carried out using the MATLAB Statistics toolbox at a significance
 541 level of 1% for peak frequencies of the 165 time windows compared. The peak frequency samples
 542 measured from video and audio were tested to check if the distributions had highly similar mean
 543 and variance, indicating that they have been sampled from the same probability distribution func-
 544 tion. This method applies to Figure 2C.

545 The Bhattacharya overlap coefficient (BC) and Jensen-Shannon Divergence metric (JSD) were com-
 546 puted between pairs of wingbeat frequency distributions, where the number of counts of wingbeat
 547 frequency in each bin are normalized by the product of the total number of samples and the range
 548 of the independent variable (frequency, 200 to 700 Hz) to yield a probability density distribution with
 549 unit area under the curve. BC is calculated as the sum of the geometric mean of the two probability
 550 densities in each bin, ranging between a minimum of 0 for disparate non-overlapping distributions
 551 and a maximum of 1 for identical distributions. The JSD is calculated as the square root of the
 552 arithmetic mean of the Kullback-Leibler divergences of each distribution with respect to the other,
 553 for each pair of distributions considered. These methods apply to Figs. 2G,H, and Figure 3B.

554 **Testing and validation of Maximum Likelihood Estimation (MLE) algorithm**

555 **Algorithm description**

556 In this classification algorithm, the different species are treated as discrete values of a parameter
 557 that alters the point mass function for the distribution of frequency from mosquito sound. A given
 558 observation is statistically compared to each distribution to deduce which one it is most likely to
 559 have been sampled from, and the species with maximum likelihood to be the parent distribution
 560 is identified. We chose this strategy as an appropriate method for identification, as it attempts to
 561 identify the statistical distribution that is most likely to have produced the observed data, i.e. it
 562 attempts to identify the species that is most likely to have produced an observed sound recording.
 563 This is particularly appropriate as we treat each recording not as a single data point, but as a distri-
 564 bution of wingbeat frequencies over the duration of the recording. Hence, to compare and classify
 565 the observed distribution from the recording with respect to the various reference distributions,
 566 MLE provides a natural solution.

567 We use wingbeat frequency data from each species, isolated for each 20 ms sample window using a
 568 peak finding routine on the spectrogram as described earlier, to construct the point mass functions
 569 that describe the wingbeat frequency distributions for each species. We also treat any given acoustic
 570 recording being queried as a collection of discrete samples of wingbeat frequency from 20 ms
 571 windows. For each sample window frequency in this recording, we find the probability density of
 572 its occurrence in the frequency distributions corresponding to each species. We thus construct a
 573 matrix that gives the probability of occurrence in each of the frequency distributions corresponding
 574 to each species, for each sample point of frequency in the given recording. Assuming each sample
 575 point of frequency in the given recording to be independent, we now sum across all sample points
 576 to obtain a measure of the combined likelihood that all sample window frequencies in the recording
 577 were produced from each species frequency distribution. We add these values instead of multiply-
 578 ing, to avoid there case where a single outlier point has the power to veto a certain species due to
 579 having zero probability of occurrence in the corresponding frequency distribution. We choose the
 580 species which has the maximum likelihood for the total over all sample windows of that recording,
 581 as the species with the maximum likelihood of having produced a recording with that given set of

582 frequency values.

583

584 Location filter

585 We constructed a location filter by looking up the presence or absence of all the species in our
586 database in a set of 200 countries, as recorded in the Walter Reed Biosystematics Unit Mosquito
587 Identification Database (*Walter Reed Biosystematics Unit, 2017*). When applying the filter, we
588 choose a location at random for a recording from a given species, based on its geographical distri-
589 bution as specified in our location matrix. When finding the species with the maximum likelihood
590 of having produced the frequencies observed in that recording, we now only choose from other
591 species that are also located in the country chosen for our recording, as per our location matrix.
592 This winnows down the number of choices for the species, eliminating those with similar frequency
593 distributions but which are not geographically relevant.

594

595 Validation using bootstrapped data

596 We first applied our algorithm to find the probability of correct classification of bootstrapped data
597 from the original set of frequency values that was used to generate the wingbeat frequency distribu-
598 tions for each species. This is expected to correctly classify species whose frequency distributions
599 do not have much overlap with others, and make mistakes when classifying species which have
600 significant overlaps with others. For each species, we choose a random subset of frequency values
601 from the original distribution and assign it to the species with maximum estimated likelihood. We
602 repeat this for a total of 10,000 random subsets per species, to find the asymptotically convergent
603 probability of classification for a large number of trials. A confusion matrix was generated by finding
604 the fractions of these 10,000 trials for each species that were classified correctly, or erroneously to
605 a certain other species. As expected, we see very high classification accuracies (greater than 0.99)
606 for species with relatively non-overlapping frequency distributions, with errors arising exclusively in
607 the classification of overlapping species.

608

609 K-fold validation by partitioning data

610 To get an idea of classification errors that arise when the data being queried is not itself used to
611 generate the distribution against which it is being compared, we performed K-fold validation for K
612 from 2 to 10. Here, we partitioned the data into K groups, and used one group to generate queries
613 which were matched against distributions generated using the other (K-1) folds. The confusion
614 matrix generated in this case had very similar values as the one generated for bootstrapped data.

615

616 Validation using test data

617 We finally validated our approach against test data that was collected in a separate experiment
618 using a different phone, from the same reference populations. Contiguous individual mosquito
619 traces were chosen from these recordings, and compared against the reference distributions. This
620 validation was performed both with and without the location filter, to show the effect of including
621 location metadata to refine classification.

622

623 Mosquito specimens

624 Mosquito colonies were sourced from a number of different labs and facilities, including our own.
625 Mosquitoes were typically chosen to be between 5 to 15 days, with all individuals in a colony aged
626 within two days of each other. Females were typically mated but not bloodfed.

627 The lab reared colonies used in this work sourced from the BEI Resources Vector Resource collection
628 were *Aedes aegypti* (strain COSTA RICA, provided by the Animal Flight Lab at UC Berkeley), *Aedes*

629 *aegypti* (strain NEW ORLEANS, reared at CDC, Atlanta), *Aedes aegypti* healthy and infected with *D.*
 630 *immitis* (strain NEW ORLEANS, provided by the Zohdy Lab at Auburn University), *Aedes albopictus*
 631 (strain ALBOPICTUS, reared at CDC, Atlanta), *Anopheles albimanus* (strain STECLA, reared at CDC,
 632 Atlanta), *Anopheles arabiensis* (strain DONGOLA, reared at CDC, Atlanta), *Anopheles arabiensis* (strain
 633 RUFISQUE, reared at CDC, Atlanta), *Anopheles atroparvus* (strain EBRO, reared at CDC, Atlanta),
 634 *Anopheles dirus* (strain WRAIR2, reared at CDC, Atlanta), *Anopheles farauti* (strain FAR1, reared at CDC,
 635 Atlanta), *Anopheles freeborni* (strain F1, reared at CDC, Atlanta), *Anopheles gambiae* (strain KISUMU,
 636 reared at CDC, Atlanta), *Anopheles gambiae* (strain AKRON - bendiocarb resistant, reared at CDC,
 637 Atlanta), *Anopheles gambiae* (strain RSP - permethrin resistant, reared at CDC, Atlanta), *Anopheles*
 638 *merus* (strain MAF, reared at CDC, Atlanta), *Anopheles minimus* (strain MINIMUS1, reared at CDC,
 639 Atlanta), *Anopheles quadriannulatus* (strain SANQUA, reared at CDC, Atlanta), *Anopheles stephensi*
 640 (strain STE2, reared at CDC, Atlanta), *Anopheles stephensi* (strain STE2, provided by the Luckhart Lab
 641 at UC Davis), *Culex quinquefasciatus* (strain JHB, reared at CDC, Atlanta), *Culex tarsalis* (strain Yolo,
 642 reared by us).

643 Colonies caught in the field or bred from catches included *Aedes aegypti* (F1, Los Angeles, provided
 644 by the Coffey Lab at UC Davis), *Aedes aegypti* (F1, Puerto Rico, provided by the Coffey Lab at UC
 645 Davis), *Aedes mediovittatus* (F0, provided by the Coffey Lab at UC Davis), *Anopheles quadrimaculatus*
 646 (F22, Alabama, provided by the Mathias Lab at Auburn University), *Culex pipiens pipiens* (provided
 647 by the Santa Clara Vector Control District), *Culex pipiens pipiens* (provided by the San Mateo Vector
 648 Control District), *Culex quinquefasciatus* (provided by the San Mateo Vector Control District). Wild
 649 mosquitoes captured by us in field trials include *Culiseta incidens* (captured at Jasper Ridge Biological
 650 Preserve, Stanford University, and in San Francisco), *Ochlerotatus sierrensis* (captured at Big Basin
 651 Redwoods State Park, California, USA), *Anopheles spp.*, *Culex spp.* and *Mansonia spp.* (captured at the
 652 Centre ValBio and Ranomafana village, Madagascar).

653 **Comparisons with high speed videography**

654 We acquired high speed video of tethered mosquitoes in the lab using a Phantom v1610 camera, at
 655 10000 frames per second. Simultaneously, we made audio recordings using a mobile phone placed
 656 with the primary microphone 10 mm away and oriented towards the mosquito. Since the audio
 657 and video are completely independent as the camera does not talk to the phone, synchronization
 658 was achieved using a specially designed setup to produce a specific light and sound pattern. We
 659 connected a piezoelectric buzzer and an LED to the same pin of an Arduino. The buzzer and LED
 660 are both fed the same pattern of voltage by the microprocessor. Hence the frequency of sound
 661 from the buzzer recorded in the audio exactly matches the frequency of light flashes from the
 662 LED recorded in the video at every instant of time, allowing the audio and video spectrograms
 663 to be aligned in time. We programmed the Arduino to produce a square wave at 5000 Hz and
 664 50% duty cycle for 500 ms, followed by a 500 ms pause, and then a square wave at 2000 Hz for 500
 665 ms. This gave us four time points - the beginning and end of each waveform - to use for aligning
 666 the corresponding spectrograms from video and audio in time. The video data was thresholded
 667 and the area of the wing (which we recorded face on) was computed in each frame. We plotted
 668 a waveform of the change in projected wing area over time, and applied the STFT to produce a
 669 spectrogram. The fundamental frequency in the spectrogram corresponded to the wingbeat, with
 670 higher harmonics corresponding to subtler variations in wing kinematics such as wing deformation
 671 during clap-and-fling. Acoustic data from the mobile phone was processed as described in the
 672 section above. The two spectrograms were computed to the same time and frequency resolutions
 673 of 5 Hz and 20 ms, and aligned in time based on the best match of the four points of synchronization.
 674 This method applies to Figure 2B.

675 **Comparisons to studio microphones**

676 Comparison with an acoustic gold standard was achieved using the Marshall MXL991 and Apex 220
 677 microphones, the latter of which is calibrated to have a flat frequency response between 100 and

678 1000 Hz. The two studio microphones were connected to a pre-amplifier (Onyx) with the gain set to
 679 its maximum value of -60dB , after ensuring that this would still avoid saturation. We carried out
 680 experiments to calibrate the sensitivity of mobile phones over distance using a standardized sound
 681 source - a piezoelectric buzzer ringing at 500 Hz, with its amplitude measured before every recording
 682 to be constant at 77dB at the edge of the buzzer disc. To compare the ability of mobile phones to
 683 record mosquito sounds, we recorded tethered male and female *Culex tarsalis* mosquitoes. We
 684 placed the microphones at an identical distance to the left of the mosquito as we placed the mobile
 685 phone primary microphone to its right, since waveforms produced by the two wings are assumed to
 686 be symmetrical. We synchronized recordings from all three sources using the times of initiation and
 687 cessation of wingbeat sound, with multiple flight traces in a single dataset. Using the amplitudes
 688 recorded by the Marshall MXL991 studio microphone which has a known $1/r^2$ drop in recorded
 689 amplitude, we deduced the actual amplitude produced by the mosquito in a given experiment,
 690 and standardized the corresponding mobile phone SNR for a uniform source amplitude of 45dB
 691 (which we measured to be a typical amplitude produced by a mosquito). Spectrograms constructed
 692 independently and aligned in time are shown in Figure 1 - figure supplement 1. These methods
 693 apply to Figure 2D,E and Figure 1 - figure supplement 1.

694 **Acoustic data collection in the lab from tethered mosquitoes**

695 Individual sound traces for distance calibration experiments were collected from tethered mosquitoes.
 696 Individuals were aspirated out of the cage and knocked out with a puff of carbon dioxide. The wings
 697 were gently spread to move them out of the way, and a pipette tip was affixed to the scutum with
 698 a bead of low melting insect wax. The pipette tip was clamped in a stand, and the appropriate
 699 recording device - mobile phone, studio microphone or high speed camera - was clamped in another
 700 stand at the desired orientation and a specified distance away from the pipette tip as measured
 701 by a ruler. The legs of the mosquito are gently stimulated to induce a flight reflex, after which the
 702 wings beat for a period of a few seconds to minutes. This method applies to Figure 2A,B,D,E.

703 **Acoustic data collection in the lab from caged populations**

704 Wingbeat frequency distributions for a given species were measured from lab-reared populations
 705 maintained in 1-ft cubical cages. Cages typically contained between 100 to 300 individuals of males
 706 and females each, with the sexes segregated into separate cages whenever possible. The mobile
 707 phones were inserted by hand through the stockinette sleeves of the cages, with the primary
 708 microphones oriented away from the hand, and moved to follow individual mosquitoes in flight
 709 or against walls or corners of the cage. Care was taken to avoid introducing noise from bumping
 710 against the cage surfaces or rubbing against the sleeve. Between 5 to 10 minutes of data was
 711 collected per cage, and high amplitude noise due to bumps was eliminated using an automated
 712 algorithm. This method applies to Figure 2F, Figure 3A and Figure 1 - figure supplement 2. In some
 713 cases, individual mosquitoes were introduced into an otherwise empty cage, to record free flight
 714 traces from a specific mosquito in the lab. This method applies to Figure 4A,B.

715 **Acoustic data collection in the field**

716 Field acoustic data was collected in a variety of locations, including homes and gardens around
 717 San Francisco and Palo Alto, USA, at Stanford University's Jasper Ridge Biological Preserve, and
 718 around the Centre ValBio in Ranomafana, Madagascar. Mosquitoes were either followed with a
 719 phone during free flight around the user, or captured live in a Ziploc bag and subsequently recorded
 720 by putting the phone's primary microphone against the bag (while taking care not to introduce
 721 noise due to crumpling or brushing the bag surface). This method applies to Figure 4. The pilot
 722 demonstrations of field recording and mapping of mosquitoes were organized with small teams
 723 of volunteers working in pairs, with 14 and 10 users respectively in Big Basin Redwoods State Park
 724 in California, USA (20 km^2 , between 5.30 to 8.30PM on 17 August 2016), and in Ranomafana village,
 725 district Ifanadiana, Madagascar (4 km^2 , between 6PM and 8PM on 26-28 October 2016). During the

726 studies, volunteers were hiking along trails in Big Basin Park, and were gathered in houses or shops
727 in Ranomafana village. Prior to the field study, we initially collected live mosquitoes from the field,
728 recorded them in the lab to create a curated database of signatures for those specific locations,
729 and later morphologically identified them through microscopy for association with each acoustic
730 signature (Figure 4A,B). Subsequently, field recordings were made by the teams, and each recording
731 was assigned a species by comparing with the databases (Figure 4B). During the field exercises, the
732 users also collected matched physical specimens from the field in grinder tubes and Ziploc bags
733 corresponding to many of the audio recordings, which were morphologically identified to confirm
734 the IDs assigned based on the acoustic database. The recordings were associated with a location
735 as reported by GPS or the user, and timestamped automatically by the recording application on
736 the mobile phone, for spatio-temporal placement of each observation. Maps were prepared by
737 counting the number of reliably identified acoustic signatures for each location. This method
738 applies to Figure 4C,D.

739 **Figures**740 **Figure 1. Mobile phone users can collect acoustic data from mosquitoes character-**
741 **ized by the base frequency and harmonics.**

742 **A**, Illustration showing the collection of mosquito acoustic data by mobile phone users in different
743 locations. **B**, Methods to acquire wingbeat sounds from mosquitoes using mobile phones include
744 lab methods like (i) collecting them in cages, and field methods like (ii) following mosquitoes in
745 free-flight, or (iii) capturing them in cups, bottles or inflated bags. **C**, Spectrogram for a flight trace
746 acquired from an individual female *Anopheles gambiae* mosquito using a 2006 model Samsung SGH
747 T-209 flip phone. The wingbeat base frequency at every instant is computationally identified and
748 marked with a black line. (Top) The time-averaged spectrum of this flight trace shows the distribution
749 of acoustic power among the base frequency and multiple harmonics. **D**, The variations in wingbeat
750 base frequency of the mosquito during this flight trace are represented by a probability distribution
751 of the frequency identified in each window of the spectrogram. (Top) Raw base frequency data is
752 represented as a violin plot with an overlaid box plot marking the inter-quartile range, black circle
753 representing mean frequency, gray vertical bar for median frequency, and whiskers indicating 5th
754 and 95th quantiles.

755 **Figure 1 - figure supplement 1. Schematic of proposed surveillance system using**
756 **crowdsourced acoustic data from mobile phones**

757 System architecture showing the collection of data by individual mobile phone users, processing to
758 identify species of interest, and compilation into a map of mosquito activity. The diagram is depicted
759 centering around data collection at a field site designated Location X. **A-D** occur prior to mobile
760 phone based data collection, and represent steps required to enable acoustic mosquito surveillance
761 at the field location. **A**, The mosquito population in the field at Location X is sampled, either by
762 users in Ziploc bags or by using methods such as trapping, and live specimens characteristic to the
763 location are collected. **B**, Wingbeat sounds of these field collected mosquitoes are recorded, with an
764 acoustic dataset associated with each individual specimen. **C**, Specimens are identified to the genus
765 (and preferably species) level by a method such as morphological ID through optical microscopy,
766 or molecular ID through PCR. **D**, Acoustic data is processed and associated with specimen IDs to
767 yield frequency distributions characteristic of the prevalent species in that field location, forming a
768 reference database of mosquito sounds specific to Location X. **E-H** represent the proposed method
769 for mobile phone based acoustic surveillance at the field location, assuming that the reference
770 database of mosquito sound is already in place. **E**, Mosquitoes are recorded in the field by a
771 user with a mobile phone, and the audio file together with metadata is compiled into a database
772 for processing. **F**, The acoustic signals are processed to extract the frequencies present in the
773 recorded mosquito sound. **G**, The computed acoustic spectrum and metadata obtained from
774 the mobile phone are compared to the reference database for that location, and the most likely
775 species corresponding to the computed frequency is identified. **H**, The identified species from this
776 observation, together with the time and location metadata, are mapped back to the field Location
777 X. This closes the loop for mobile phone based acoustic surveillance, from user recorded data to
778 information on spatio-temporal mosquito activity.

779 **Figure 1 - figure supplement 2. Brochure**

780 **A**, Graphical brochure / poster showing techniques for recording acoustic data from mosquitoes, to
781 train users from the general public.

782 **Figure 2. Mobile phones sensitively acquire high fidelity acoustic data from mosquitoes**
 783 **with comparable performance across models**

784 **A**, Schematic of experimental setup for recording a tethered mosquito using synchronized mobile
 785 phone audio and high speed video. Synchronization between audio and video is achieved on the
 786 order of microseconds, using a piezoelectric buzzer connected in parallel with an LED, and controlled
 787 with a microprocessor to produce the same temporal sequence of sound and light. **B**, Overlaid
 788 spectrograms for female *Culex tarsalis* mosquitoes obtained independently using high speed video
 789 (magenta) and mobile phone audio (cyan), aligned to within 2 ms and showing a spectral overlap
 790 (blue) within 2 Hz across all time instances. The mobile phone data is noisy but faithfully reproduces
 791 the base frequency peak of 264 Hz and the first two overtones. **C**, Base frequency distributions
 792 from video and audio (165 time instances each) have high overlaps, as measured by a Bhattacharya
 793 Coefficient (BC) of 0.95. **D**, Signal-to-noise ratio (SNR) estimates over distance from a standardized
 794 sound source show that mobile phone microphone performance within a 100mm radius is superior
 795 or comparable to high performance studio microphones. The pink line represents the actual source
 796 amplitude, with the pink shaded region below indicating the region less than the actual amplitude,
 797 in which all acoustic measurements should lie. The gray shaded band represents the range of
 798 sensitivities between the limits set by the two studio microphones used as reference standards. The
 799 SNR curves for all three phones lie mostly within this band, indicating that they perform in a range
 800 between the two reference microphones. **E**, SNR over distance for the wingbeat sound produced
 801 by a tethered female *Cx. tarsalis* mosquito (normalized for a source amplitude of 45 dB), provide
 802 working limits where phones can detect the audio signal - 50 mm for the low end T-209 feature
 803 phone and 100 mm for the iPhone 4S and Xperia Z3 Compact smartphones. The gray dotted line
 804 represents the actual amplitude of the mosquito sound in dB, as measured by the MXL991 reference
 805 microphone. The gray shaded region below indicates the region less than the actual amplitude, in
 806 which all acoustic measurements are expected to lie. **F**, Variation of the base frequency distribution
 807 sampled by 8 different phones is low compared to the natural variation within a population of
 808 lab-reared *Anopheles stephensi* females. Raw data are shown with overlaid box plots marking the
 809 inter-quartile range, black circles for mean frequency, gray vertical bars for median frequency, and
 810 whiskers indicating 5th and 95th quantiles. **G,H**, The Jensen-Shannon divergence metric for base
 811 frequency distributions (**G**, lower left triangle) shows low disparity, ranging between 0.144 and 0.3,
 812 against a minimum of 0 for identical distributions. Likewise, the Bhattacharya distance (**H**, upper
 813 right triangle) shows high overlap, with values between 0.935 to 0.986, against a maximum of 1
 814 for identical distributions. The brown hatched areas along the diagonal represent blank cells, as
 815 distances are not shown for any distribution with respect to itself.

816 **Figure 2 - figure supplement 1. Synchronized recordings of tethered mosquitoes**
817 **using studio and mobile phone microphones shows exact correspondence at dis-**
818 **tances below 50 mm**

819 **A-E**, Comparison of power spectral density for synchronized simultaneous recordings of individual
820 *Culex tarsalis* female mosquitoes using the MXL 991 studio microphone, the Apex 220 reference
821 microphone and a mobile phone, taken at varying distances. The left column corresponds to the
822 SGH T-209 feature phone, the middle column to the iPhone 4S iOS smartphone, and the right
823 column to the Xperia Z3 Compact Android smartphone. **A**, Superimposed averaged spectra show
824 that all phones acquire wingbeat sound at a high signal-to-noise ratio at 10 mm away from the
825 mosquito. **B,C,D**, Overlaid spectrograms synchronized to within 20 ms in time show a near-perfect
826 spectral match of within 5 Hz at each time interval, for the mobile phone microphone (red channel),
827 MXL 991 (green channel) and the Apex 200 (blue channel), shown together as RGB images with
828 intensity of colour corresponding to variations in power spectral density. Mobile phones strongly
829 acquire mosquito sounds at 10 mm or even 50 mm, but their sensitivity drops sharply at distances
830 of 100 mm. **E**, Superimposed averaged spectra show that only the Xperia Z3 continues to acquire
831 wingbeat sound at 100 mm away from the mosquito, albeit at low signal-to-noise ratio. The T209
832 feature phone picks up low frequency noise between 300 to 600 Hz that overwhelms the mosquito
833 frequencies, the iPhone 4S has low noise acquisition throughout, and the Xperia Z3 picks up high
834 frequency noise above 1 kHz that leaves the mosquito frequency band relatively unaffected.

835 **Figure 3. Mosquitoes of different species are distinguishable based on base fre-**
 836 **quency distributions and metadata**

837 **A**, Distribution of base frequencies for female mosquitoes of 20 vector species, for recordings
 838 obtained with the 2006 model T-209 low-end feature phone (except *Cu. incidens*, *Cx. pipiens* and
 839 *Cx. quinquefasciatus*, recorded using iPhone models; and *Ae. sierrensis*, recorded with various
 840 phones). **B**(lower left triangle), Jensen-Shannon divergence metric for base frequency distributions.
 841 Distributions are spaced apart with high J-S divergence in most cases, with only four pairwise
 842 combinations having J-S divergence around 0.35 - the maximum divergence for the same species
 843 across different phones. **C** (upper right triangle), Qualitative classification of species pairs according
 844 to the possibility of distinguishing them using mobile phones — (i) no frequency overlaps, hence
 845 distinguishable by acoustics alone, (ii) overlapping frequency distributions, but not geographically
 846 co-occurring hence distinguishable using location, (iii) overlapping frequency distributions but
 847 distinguishable using time stamps, (iv) partially overlapping frequency distributions but no location-
 848 time distinctions, hence distinguishable but not in all cases, (v) indistinguishable due to highly
 849 overlapping frequency distributions with co-occurrence in space and time. The brown hatched
 850 areas along the diagonal represent blank cells, as distances are not shown for any distribution
 851 with respect to itself. **D**, Confusion matrix for classification of acoustic data bootstrapped from the
 852 reference frequency datasets for twenty species, using the Maximum Likelihood Estimation (MLE)
 853 algorithm. Each column corresponds to a particular species, from which acoustic data is drawn for
 854 classification. The twenty entries in each column represent the fractions in which recordings from
 855 the species corresponding to that column are classified among all twenty species in our database.
 856 This classification is done for data taken from the reference distributions, and is compared against
 857 the same reference dataset, based exclusively on wingbeat frequency. Classifications errors occur
 858 when a given species frequency distribution has overlaps with other species, and the confusion
 859 matrix reflects the inherent uniqueness or overlap between frequency distributions in our database.
 860 Gray areas along three columns represent species for which test data from a different phone was
 861 not available. Colour scale showing fraction of recordings classified is the same for both **D** and
 862 **E**. **E**, Confusion matrix for classification of test audio recordings, using the MLE algorithm. Test
 863 data was collected for 17 species, using different phones (some or all among Google Nexus One,
 864 Sony Xperia Z3 Compact, iPhone 4S) which were not used to construct the reference distributions
 865 (recorded using the SGH T-209). Each column corresponds to a particular test species, from which
 866 acoustic data is drawn for classification. The twenty entries in each column represent the fractions
 867 in which recordings from the test species corresponding to that column are classified among all
 868 twenty species in our database. Classification is based on both wingbeat frequency and a location
 869 filter, simulating the classification of randomly recorded mosquitoes of different species by users in
 870 field conditions. The resulting classification accuracies are significantly higher in this case, when
 871 compared to classification accuracies which do not consider location (Figure 3 - figure supplement
 872 1D). Blank areas along three columns represent species for which test data from a different phone
 873 was not available. **F,G**, Variations in base frequency distribution (**F**) for field-recorded sounds
 874 corresponding to wild female *Ae. sierrensis* mosquitoes having a wide (about two-fold) variation
 875 in body size and wing area (**G**), showing small differences between individuals compared to the
 876 variation within each flight trace. The gray distribution at the top represents the species wingbeat
 877 frequency distribution for *Ae. sierrensis*, with the gray shaded vertical band marking the range from
 878 5th to 95th percentile of frequency for the species. All individual recordings lie completely within
 879 this range.

880 **Figure 3 - figure supplement 1. Statistical parameters for adequate sampling, inter-**
881 **colony variations and test data classification accuracies for wingbeat frequency**
882 **distributions**

883 **A,B**, Convergence of statistical metrics for randomly sampled subsets of increasing size boot-
884 strapped from species wingbeat frequency distributions for 20 species, to values expected for
885 subsets having identical statistical parameters as the parent distribution. Colours correspond to
886 each species. The Bhattacharya coefficient (BC) (**A**) approaches 1 indicating perfect overlap of point
887 mass functions for subsets and parent distributions, while the Jensen-Shannon Divergence metric
888 (JSD) (**B**) approaches 0 indicating that both subset and parent distributions contain the same infor-
889 mation. These metrics change rapidly for subsets having few sample points, but quickly converge
890 to their expected values within a small fraction of the total number of samples comprising each
891 species frequency distribution. **C**, Distributions of wingbeat frequency for different populations of
892 the same species. *Anopheles gambiae* and *Aedes aegypti* have highly overlapping distributions for
893 different colonies, whereas *Anopheles arabiensis* shows significant difference in wingbeat frequency
894 between two variants. **D**, Confusion matrix for classification of audio recordings using the Maximum
895 Likelihood estimation algorithm, for test data from various phones, as compared to the reference
896 datasets for 20 species recorded using the SGH T209 phone. Each column shows the probability
897 of correct or erroneous classification of a given species with respect to all others, based solely on
898 wingbeat frequency. The resulting classification accuracies are comparatively lower than those
899 which factor in location metadata (Figure 3E). Blank areas along three columns represent species
900 for which test data from a different phone was not available.

901 **Figure 3 - figure supplement 2. Mosquito species can be distinguished with mobile**
902 **phone acoustics and metadata**

903 **A-F**, Illustrative examples for distinguishing between medically relevant mosquito species using
904 acoustics and metadata. All inset images of mosquito specimens are taken from Walter Reed
905 Biosystematics Unit mosquito ID databases. **A**, Distinction by acoustic data alone - *Cx. pipiens* and
906 *Anopheles gambiae*, which co-occur in many regions, can easily be distinguished by sound alone. **B**,
907 Distinction by location metadata - *An. atroparvus* and *An. dirus* have overlapping acoustic spectra, but
908 recordings are easily distinguished from each other by metadata pertaining to their distinct spatial
909 distributions in Europe and South-East Asia respectively. **C**, Possible partial distinction by time
910 metadata - *Aedes aegypti* and *An. gambiae* can occur together in many locations and have overlapping
911 wingbeat frequency distributions, but may possibly be distinguished in some instances by time
912 of recording, based on their differential preferences for diurnal and crepuscular biting. **D**, Partial
913 distinction by acoustic data - *Ae. aegypti* and *Ae. albopictus* have similar appearances, geographical
914 distributions and biting habits in many areas. Although the wingbeat frequency distributions are
915 not completely distinct, interquartile ranges do not overlap and a significant fraction of recordings
916 can still be classified correctly as one or the other, making acoustic identification faster and easier
917 than microscopy. Similarly in the case of *Cx. pipiens* and *Cx. quinquefasciatus*, which have partially
918 distinguishable frequency spectra despite being otherwise indistinguishable except using PCR. **E**,
919 Partial distinction by acoustic data - *An. arabiensis*, *An. gambiae*, and *An. quadriannulatus*, which are
920 members of a species complex that are identical in appearance and often overlapping in habitat,
921 have non-overlapping interquartile ranges for wingbeat frequency distributions implying that the
922 majority of acoustic samples can be classified correctly as one among the three. **F**, *An. arabiensis* is
923 indistinguishable based on mobile phone acoustic data from *An. merus*, another members of the
924 *An. gambiae s.l.* species complex, exposing a relatively rare limitation of species identification using
925 mobile phone acoustic surveillance.

926 **Figure 4. Spatio-temporal activity of mosquitoes in the field can be mapped using**
 927 **acoustic data collected by mobile phone users.**
 928 **A**, Sample spectrograms from female *Culex spp.* (top) and *Anopheles spp.* (bottom) mosquitoes
 929 captured in the field at Ranomafana in Madagascar. **B**, Frequency distributions for field-caught
 930 *Culex spp.* and *Anopheles spp.* mosquitoes in Ranomafana, forming a reference for identification
 931 of recordings from either species at this field site. Acoustic data was collected for 3 minutes each,
 932 from 50 individual *Culex* and 10 individual *Anopheles* mosquitoes. **C**, Map of Ranomafana village
 933 showing distribution of female *Culex spp.*, *Anopheles spp.*, and *Mansonia spp.* mosquitoes, from
 934 mobile phone data recorded by 10 volunteers over the approximately 1 km X 2 km area. Each
 935 square represents one recording, and black circles indicate locations where volunteers reported
 936 encountering no mosquitoes. The numbers in the white boxes show the number of *Culex* (pink)
 937 and *Anopheles* (gray) mosquitoes captured in CDC light traps over the same time period at those
 938 locations. The map shows a spatial gradient from riverbank to hillside in the relative proportion
 939 of *Anopheles spp.* and *Culex spp.* mosquitoes. Further, mosquito hotspots are interspersed with
 940 points having a reported lack of mosquitoes, highlighting the potential importance of factors such
 941 as the distribution of water and livestock. **D**, Spatio-temporal activity map for female *Ae. sierrensis*
 942 mosquitoes in the Big Basin Park field site, using data collected by 13 hikers recording mosquitoes
 943 with their personal mobile phones, over a 3-hour period in an approximately 4.5 km X 5.5 km area.
 944 Each brown square represents one *Oc. sierrensis* female recording, and black dots represent sites
 945 where hikers reported encountering no mosquitoes at all. (Inset top left) Temporal distribution of
 946 the overall mosquito activity data depicted in **(D)** based on recording timestamps, showing the rise
 947 and fall in the number of recordings made, a proxy for mosquito activity, in each hour of the field
 948 study.

949 **Figure 4 - figure supplement 1. Mobile phones are capable of acquiring mosquito**
950 **sounds in a variety of field environments**
951 **A-F**, Raw spectrograms of acoustic data acquired by various mobile phone users in different
952 field conditions, with base frequencies of mosquito sounds highlighted by a box. The signals
953 include sources of noise such as human speech, fire truck sirens, and birdsong, and were acquired
954 in both urban (**A-D**) and forested (**E,F**) environments, including indoor (**A,B**) and outdoor (**C-F**)
955 settings. Mosquitoes recorded were either followed in free-flight (**A,C,E**) or captured in a plastic
956 ziploc bag prior to recording (**B,D,F**). All spectrograms show raw spectra without background
957 correction or noise removal, and show the spectra from extraneous acoustic sources (speech,
958 sirens) to distinguish the characteristics of mosquito spectra from other sounds. Spectrograms **A-F**
959 correspond to sounds in Supplementary Audio SA2-7.

960 **Figure 4 - figure supplement 2. Individual flight traces for wild mosquitoes show**
961 **highly similar mean frequencies with small but intrinsic variances**
962 **A**, Distribution of wingbeat frequencies for 74 representative recordings of wild female *Aedes*
963 *sierrensis* mosquitoes from Big Basin Redwoods State Park, CA, USA. The bottom distribution
964 shows the overall species wingbeat frequency distribution for *Ae. sierrensis* aggregated from all
965 recordings, with the colour of each data point corresponding to its contributing flight trace. The
966 even distribution of the colours across frequency for the overall species distribution indicates that
967 most individual flight traces have similar frequency distributions, means and variances. **B**, Plot of
968 mean frequencies of individual flight traces against their corresponding standard deviation as a
969 percentage of the mean. The gray line indicates cumulative fraction of recordings, showing that
970 85% of recordings have mean frequencies clustered between 350 and 450 Hz, with the vast majority
971 having frequency spreads of less than 5% of the mean. **C**, Plot of duration of flight traces against
972 the relative standard deviation, coloured by the value of mean frequency, shows no correlation
973 or clustering between these characteristics. However, there appears to be a minimum spread of
974 about 2% of the mean for most flight traces, irrespective of duration or mean frequency, which
975 appears to correspond to inherent natural frequency variations within flight traces. **D**, Variation of
976 classification accuracy for flight traces with relative standard deviation and mean frequency of the
977 trace. For **D** and **E**, the colours represent the probabilities of classifying the trace as *Ae. sierrensis*
978 using the MLE algorithm. Larger dots indicate those that were correctly classified, while small dots
979 represent traces that were incorrectly classified as one of the other local species - *Cx. pipiens*, *Cx.*
980 *quinquefasciatus*, *Cx. tarsalis* or *Cu. incidens*. In **D**, the grey dashed line represents the boundary
981 between traces that were correctly and wrongly classified. Classification accuracy is observed to
982 be solely a function of mean frequency of the trace, corresponding to the overlaps of wingbeat
983 frequency distributions. **E**, Variation of classification accuracy for flight traces with relative standard
984 deviation and duration of the trace. There is no relationship observed between correct classification
985 and the recording duration or frequency spread of the trace.

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1011 Competing Interests

1012 The authors declare no competing interests.

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1122 **Supplementary Audio Files**

1123 **Supplementary Audio SA1**

1124 Sample acoustic recording from a lab-reared 5-day old female *Anopheles gambiae* mosquito in a
1125 cage at the CDC insectary, Atlanta, GA, USA. Recording was done by one of the authors, using a
1126 2006-model Samsung SGH T-209 clamshell phone sampling at 8kHz.

1127 **Supplementary Audio SA2**

1128 Acoustic recording from wild male *Culex pipiens* mosquito in a living room in Menlo Park, CA, USA.
1129 Recording was done by one of the authors using a 2010-model iPhone 4, sampling at 48kHz.

1130 **Supplementary Audio SA3**

1131 Acoustic recording from wild female *Culex pipiens* mosquito in a bathroom in Redwood City, CA, USA.
1132 Recording was done by a volunteer using a 2014-model Samsung Galaxy A3, sampling at 44.1kHz.

1133 **Supplementary Audio SA4**

1134 Acoustic recording from wild female *Culex pipiens* mosquito in a garden in Menlo Park, CA, USA.
1135 Recording was done by one of the authors using a 2010-model iPhone 4, sampling at 48kHz.

1136 **Supplementary Audio SA5**

1137 Acoustic recording from wild female *Culiseta incidens* mosquito in a garden in San Francisco, CA,
1138 USA. Recording was done by one of the authors using a 2015-model iPhone 6S sampling at 48kHz.

1139 **Supplementary Audio SA6**

1140 Acoustic recording from wild female *Ochlerotatus sierrensis* mosquito at Big Basin Redwoods State
1141 Park, CA, USA. Recording was done by a volunteer using a 2015-model Sony Xperia Z3 compact,
1142 sampling at 44.1kHz.

1143 **Supplementary Audio SA7**

1144 Acoustic recording from wild female *Culiseta incidens* mosquito near a highway at Jasper Ridge
1145 Biological Preserve, CA, USA. Recording was done by a volunteer using a 2006-model Samsung SGH
1146 T-209 clamshell phone sampling at 8kHz.

1147 **Supplementary Audio SA8**

1148 Acoustic recording from wild female *Anopheles spp.* mosquito near an outdoor pig pen in Ra-
1149 nomafana, Madagascar. Recording was done by a volunteer using a locally available non-smart
1150 phone sampling at 44.1kHz.

1151 **Supplementary Audio SA9**

1152 Acoustic recording from wild female *Culex spp.* mosquito inside a local residence in Ranomafana,
1153 Madagascar. Recording was done by a volunteer using a locally available non-smart phone sampling
1154 at 44.1kHz.

1155 **Supplementary Audio SA10**

1156 Acoustic recording from wild female *Aedes sierrensis* mosquito at Big Basin Redwoods State Park,
1157 CA, USA. Recording was done by a volunteer using a 2015-model HTC-One M8 phone sampling at
1158 44.1kHz.

1159 **Supplementary Video Files**

1160 **Supplementary Video SV1**

1161 Demonstration of how to identify the primary microphone of a mobile phone and record sound
1162 from a mosquito confined in a cup.

1163 **Supplementary Video SV2**

1164 Demonstration of how to record sound from a mosquito confined in a bottle, using a mobile phone.

1165 **Supplementary Video SV3**

1166 Demonstration of how to record sound from a mosquito in free flight, at the moment of take-off,
1167 using a mobile phone.

1168 **Supplementary Video SV4**

1169 High speed video recording from a lab reared female *Culex tarsalis* mosquito, tethered by the
1170 scutum to a pipette tip using a bead of wax. The calibration LED visible at the bottom left is used to
1171 automatically synchronize time points in the video with corresponding beeps from a piezoelectric
1172 buzzer in the audio. Recording was done on a Phantom v1610 high speed camera sampling at
1173 10kHz.

1174 **Downloadable data package**

1175 As part of our policy to openly share all data from this project, we have included a downloadable
1176 package comprising all acoustic data collected over the course of this work. This includes acoustic
1177 recordings from 20 different species of mosquitoes, using a variety of mobile phones for each. This
1178 data can be downloaded either from our project website at abuzz.stanford.edu, or from the online
1179 repository at Dryad (<http://dx.doi.org/10.5061/dryad.98d7s>). The supplementary audio files are
1180 not included in this package, and may be downloaded separately.