Do hoverflies (Diptera: Syrphidae) sound like the Hymenoptera they morphologically resemble?

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It has long been recognized that many hoverfly species (Diptera: Syrphidae) mimic the morphological appearance of defended Hymenoptera, such as wasps and bees. However, it has also been repeatedly suggested that some mimetic hoverflies respond with sounds on attack that resemble the warning or startle sounds of their hymenopteran models. In this study, we set out to quantitatively compare the spectral characteristics of the sounds produced by a range of nonmimetic flies, wasps, bumblebees, honeybees, and their hoverfly mimics when they were artificially attacked. The sounds made by wasps and honeybees after simulated attacks were statistically distinguishable from their hoverfly mimics. Bumblebee models of their hoverfly mimics share some similarities in the sound they produce on attack, but they were no closer acoustically to their model than a range of other hoverfly species that morphologically resemble other models. All the mimetic hoverflies tested in this study tended to sound similar to one another, regardless of the model they resemble morphologically. Overall, we found little evidence that mimetic hoverflies sound like their hymenopteran models on attack, and we question whether acoustic mimicry has evolved in this complex. *Key words:* acoustic mimicry, Batesian mimicry, Hymenoptera, startle, Syrphidae. *[Behav Ecol 20:396–402 (2009)]*

Batesian mimicry (Bates 1862) occurs when members of a palatable species (the "mimic") gain a degree of protection from predators by visually resembling an unpalatable or otherwise defended species (the "model," for reviews, see Edmunds 1974; Ruxton et al. 2004). Some of the best examples of Batesian mimicry occur in hoverflies (Diptera: Syrphidae), a group that have evolved to mimic a variety of Hymenoptera including wasps, bumblebees, and honeybees (e.g., Mostler 1935; Dittrich et al. 1993; Gilbert 2005; see Figure 1). However, mimicry in this group is not just limited to morphological similarity. For instance, some hoverflies (e.g., *Spilomyia sayi* and *Spilomyia longicornis*) wave their foreleg tibia in front of their heads to resemble the long antennae of Hymenoptera. Some species (e.g., *Spilomyia* spp.) wag their wings to resemble the wing movement of a wasp, whereas other species (e.g., *S. longicornis*, *Chrysotoxum cautum*, and *Temnostoma* spp.) pretend to sting when grabbed (see Waldbauer 1970, 1988).

One of the puzzling, yet poorly researched, behaviors exhibited by many mimetic hoverflies is their tendency to make a distinct sound when attacked or held by a bird or a potential predator (Mostler 1935; Sotavalta 1963; Brower and Brower 1965; Waldbauer 1988). Many Hymenoptera likewise appear to produce a distinct sound either during an attack or preceding it, although there is considerable variation in the nature of sounds produced and how they are transmitted. For instance, Kirchner and Röschard (1999) reported a hissing sound in the bumblebee *Bombus terrestris* in response to disturbance and/or high levels of CO_2 in the nest. Similarly, Sarma et al. (2002) showed that honeybees, *Apis florea*, use a series of hissing and "piping" behaviors in response to po-

tential predators. Whether they function as a warning signal and/or a startle to would-be predators, the sounds made by Hymenoptera appear to enhance the producer's chances of survival.

Assuming that the sounds made by Hymenoptera do function as some form of warning signal, then, like many natural signals, they may be open to exploitation (Ruxton et al. 2004). Indeed, it has been repeatedly proposed that the sounds made by mimetic hoverflies specifically resemble the sounds of their Hymenoptera models. For example, Myers (1935) noted that the spider, *Epeira diadema*, refrained from attacking the honeybee mimic hoverfly, *Eristalis tenax*, in response to its buzz, although it readily consumed nonmimetic flies. Gaul (1952) compared the frequencies of the wing beat tones of the wasp mimic hoverfly, *Spilomyia hamifera*, and the wasp, *Dolichovespula arenaria*, and reported a close similarity between the mimic and the model. Naturally, however, this similarity may have arisen entirely by chance (Gaul 1952), a hypothesis that is difficult to refute on the basis of just 2 sampled species. In a more recent account, Waldbauer (1988) described wasp-like hoverflies, stating that " ... if the fly is grasped in the fingers, or presumably by the beak of a bird, it makes a loud sound that is almost identical to the squawk of a disturbed wasp" (p. 124), but gave no formal justification for this assertion.

In this study, we test the hypothesis that on attack, hoverflies that resemble wasps and bees will acoustically mimic the hymenopteran models they morphologically resemble.

MATERIALS AND METHODS

Species investigated

The sounds produced by a total of 162 individuals from 14 different insect species were measured, analyzed, and compared (Table 1). The majority of the specimens were collected between 28 June and 15 September 2005 at the Queens University Biological Station, Ontario, Canada (44°34′N, 79°15′W).

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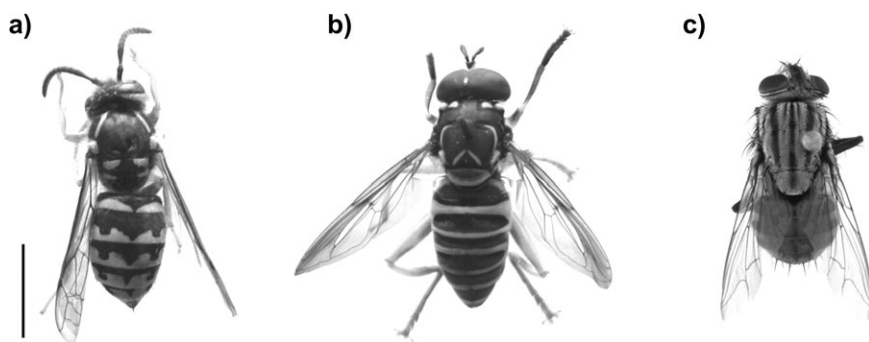
Figure 1
Some of the species investigated in our study: (a) Wasp model, *Dolichovespula arenaria*, (b) A hoverfly mimic, *Spilomyia longicornis*, and (c) A nonmimetic fly, *Sarcophaga* spp. Scale bar is 5 mm.

The exceptions were bumblebees, *Bombus impatiens* Cresson, which were reared in the laboratory, and the wasp species, *Vespula germanica* Fabricius, and honeybees, *Apis mellifera* Linnaeus, which were collected from a residential area in Ottawa, Canada. Honeybee mimics, *Eristalis transversa* Wiedemann and *Eristalis arbustorum* Linnaeus, were collected from Ottawa's Central Experimental Flower Garden. Specimens were collected using hand nets and on capture were placed in wooden and mesh cages measuring $30 \times 30 \times 50$ cm. Insects were transported back to the laboratory where acoustic recordings were made. Insects were typically tested within 2 h of capture but never more than 4 h after capture.

In total, data from an additional 18 specimens (2 *S. longicornis*, 7 honeybees, 5 *Bombus* sp., 3 *E. arbustorum*, and 1 *Sarcophaga* spp.) had to be discarded because they did not produce distinguishable or measurable sounds and therefore were not included in the analyses. Voucher specimens of 11 tested species are stored at the Canadian National Collection of Arthropods and Insects, Ottawa, Ontario, Canada (voucher numbers available on request). Example Mp3 files of the sounds made by a selection of specimens on simulated attack, and high-speed video clips indicating how these sounds are produced, are available in the accompanying Supplementary Information (Supplementary material).

Behaviors associated with sound production

We observed the mechanism of sound production on simulated attack by filming the response of a typical bumblebee, *Bombus* sp., and a wasp mimic hoverfly, *S. longicornis*, using a high-speed digital camera (2000 frames per second, Fastcam PCI HSI High-speed Imaging Incorporated, Ontario, Canada) and simultaneously recording sounds using an Earthworks QTC40 microphone. Videos and accompanying sounds were digitized and analyzed using Midas software (Xcitex, Cambridge, MA).

Acoustic recordings

Prior to audio recording, insects were dorsally tethered at the mesosternum to a 2.5-cm length of 250-μm-diameter (30 AWG) stainless steel wire (A-M Systems, Sequim, WA) using a small drop of hair removal wax. The stainless steel wire was held in a chuck-type pin holder attached to a micromanipulator, which allowed specimens to be positioned reliably in front of the microphone.

All sound recordings were performed in a walk-in–type acoustic chamber (Eckel Industries, Cambridge, MA; Model C-14A MR) measuring $2.4 \times 2.4 \times 2.4$ m. The chamber temperature was 27.0 ± 2.0 °C. A Sony stereo electret microphone (Sony, Tokyo, Japan; Model ECM-M5957) (response 100 Hz to 18 kHz) was positioned 8 cm above the insect at a 45° angle. Sounds were stored on either a Sony DAT recorder (Sony, Tokyo, Japan; Model PCM-M1) (used for all the *Sarcophaga* spp.) or a Marantz Professional digital recorder (Model PMD671) both digitizing at a rate of 48 kHz. Recording levels were the same for all specimens on the respective recorders. Sound signals recorded by the Marantz recorder were stored directly on compact flash cards in PCM-WAV format. Signals recorded on the Sony DAT were transferred to the Marantz recorder by replaying the sounds in real time and recording the analog output from the Sony DAT recorder with the Marantz recorder.

Table 1
Sound characteristics emitted by each species after attack

Groups	Species	Observations	Fundamental frequency (Hz)	f_{6L} (Hz)	f_{6R} (Hz)	F2 frequency (Hz)	diffdB
Wasp	*Dolichovespula arenaria*	18	171.6 (2.6)	164.7 (2.4)	180.0 (2.5)	345.7 (5.0)	−1.9 (0.8)
	Vespula germanica	21	152.7 (2.8)	145.3 (2.8)	158.0 (2.9)	304.9 (5.4)	3.5 (1.2)
	Vespula vulgaris	7	159.8 (5.0)	155.01 (3.8)	168.1 (5.7)	320.8 (9.6)	5.2 (3.3)
Wasp mimic	*Spilomyia longicornis*	9	290.2 (18.5)	269.5 (18.1)	304.0 (16.3)	576.9 (41.0)	11.8 (0.7)
	Helophilus fasciatus	7	291.4 (25.6)	277.8 (27.1)	306.5 (30.9)	579.1 (51.8)	14.0 (1.9)
	Spilomyia sayi	7	282.8 (11.7)	269.0 (12.5)	297.1 (11.8)	576.6 (28.5)	17.3 (3.2)
Honeybee	*Apis mellifera*	16	164.1 (11.4)	158.0 (11.1)	173.4 (11.4)	336.1 (21.8)	4.2 (1.1)
Honeybee mimic	*Eristalis transversa*	5	289.1 (17.8)	270.6 (13.5)	296.7 (16.3)	574.4 (33.2)	9.1 (3.2)
	Eristalis arbustorum	17	300.5 (11.4)	284.3 (10.9)	318.5 (13.8)	567.9 (27.1)	1.4 (1.5)
Bumblebee	*Bombus* sp.	4	263 (19.1)	257.0 (19.4)	276.9 (23.0)	521.3 (45.1)	8.8 (2.5)
	Bombus impatiens	12	316.6 (13.2)	299.6 (13.8)	328.3 (13.4)	636.8 (25.9)	9.8 (1.4)
Bumblebee mimic	*Eristalis flavipes*	10	260.6 (16.3)	247.8 (16.8)	271.7 (17.0)	519.8 (32.6)	10.2 (1.9)
Nonmimetic flies	*Chrysops relictus*	7	152.2 (8.2)	148.2 (8.1)	157.3 (8.1)	306.3 (16.0)	11.5 (1.2)
	Sarcophaga spp.	22	177.1 (6.0)	166.2 (4.8)	187.1 (6.6)	357.4 (13.1)	1.9 (0.9)

Standard errors of the means are shown in parentheses.

Tethered specimens were harassed by gently squeezing their abdomens using a pair of soft forceps, as if clasped by a bird. We made every effort to apply this tactile stimulus consistently in duration and pressure between specimens. All the attacks were directed toward the ventral side of the abdominal segments of the tethered specimens to avoid touching the wings. After recordings were complete, insects were measured in length (from head to the tip of the abdomen) using Vernier calipers to ensure that they were within the same size range of 11–14 mm.

Sound signal analysis

Spectral analysis of postattack sounds was performed using ScopeDSP software (v. 3.6a, Iowegian International, Shawnee Mission, KS, www.iowegian.com) employing an arbitrary n algorithm for calculating discrete Fourier transforms. No window function was applied to the temporal domain prior to transformation. Segments of the recordings selected for analysis were variable in length (range 426–1128 ms). The first peak in the spectrum was measured as the fundamental frequency. A second peak (F2) often occurred as a harmonic of the fundamental frequency, which was also measured. We used the difference between the peak energy at the fundamental frequency and the peak energy at its harmonic as one of the variables in our analysis (diffdB). Finally, the bandwidth of the fundamental frequency was measured as the low and high frequencies occurring 6 dB below peak; these values were designated f_{6L} and f_{6R}, respectively (measured variables are illustrated in Figure 2). We measured the bandwidth at 6 dB below peak because this represents those frequencies with half the energy of the peak.

We used SPSS version 15 (SPSS, Chicago, IL) to perform a discriminant function analysis (DFA) of the sounds produced

on response to attack. This analysis generates a set of discriminant functions based on linear combinations of continuous predictor variables that provide the most effective ways to distinguish among the known species and thereby renders a measure of the overall similarity among them (based on a Mahalanobis distance between centroids and classification probabilities of the sounds made by each and every specimen into the 14 different species). Due to the collinearity among predictors, our DFA was applied using an iterative (forward) stepwise algorithm, based on significance probability (entry $P < 0.05$, removal $P > 0.1$). Fundamental frequency and diffdB were the least correlated of all our dependent variables and were retained in the fitted model, whereas the other variables were removed. Due to the collinearity, f_{6L} and f_{6R} were removed from the final model whether they were introduced as separate variables or as a composite index of bandwidth ($f_{6R} - f_{6L}$). Comparisons of the sounds made by specimens during flight and in response to attack were conducted using matched paired t-tests, which necessarily involved a (slightly) reduced subset involving specimens that gave measurable data from both activities.

To complement the above analyses, we used multivariate analysis of variance (MANOVA) to test for population differences in the 2 least correlated sound characteristics (peak frequency [PF] and diffdB) among species. For significant MANOVA, we performed post hoc analysis (Tukey honestly significant difference) to test for population differences between individual species.

RESULTS

Behaviors associated with sound production

All individuals of the wasp-like (*S. longicornis*, *S. sayi*, and *Helophilus fasciatus*), bumblebee-like (*Eristalis flavipes*), and honeybee-like (*E. arbustorum* and *E. transversa*) hoverflies produced sounds on attack that were clearly distinct from their flight sounds (Tables 1 and 2; Figure 3), having approximately 1.5 times higher peak frequency. This elevated response was highly predictable with overall 93.8% of the recorded mimetic hoverflies (*S. longicornis*, 78%; *H. fasciatus*, 100%; *S. sayi*, 100%; *E. arbustorum*, 85%, *E. transversa*, 100%; and *E. flavipes*, 100%) exhibiting this intense buzzing reaction. The distinct high-pitched acoustic response to attack had a significantly (matched pair t-tests for each hoverfly species, all $P \leq 0.006$) higher mean fundamental frequency (260–330 Hz, Table 1) compared with the fundamental frequency of the same individual's sounds recorded during flight (140–220 Hz, Table 2; see Figure 4). High-speed imaging revealed that during this sound production period, wings were held almost parallel to the longitudinal axis of the body and thoracic segments appeared to be vibrating (see Supplementary Information, Supplementary material). The same behavioral response was observed in bumblebee species, *Bombus* sp. and *B. impatiens*, immediately after the simulated attacks.

Unlike hoverflies (see above) and bumblebees (matched pairs t-test for both species $P \leq 0.007$), the fundamental frequency of the sounds produced by nonmimetic flies, wasps, and honeybees after simulated attacks were not statistically different from sounds recorded during their flight (matched pairs t-tests, all $P > 0.05$; Figures 3 and 4). Instead of making sounds, all the individuals of the 3 social wasp species *Vespula vulgaris*, *D. arenaria*, and *V. germanica* simply tried to fly away and sting as soon as they were grasped with forceps. Likewise, both of the nonmimetic fly species investigated, *Sarcophaga* spp. and *C. relictus*, tried to fly away as soon as they were approached.

Almost all the honeybees lost their sting during handling, but they continued to perform the stinging behavior by moving
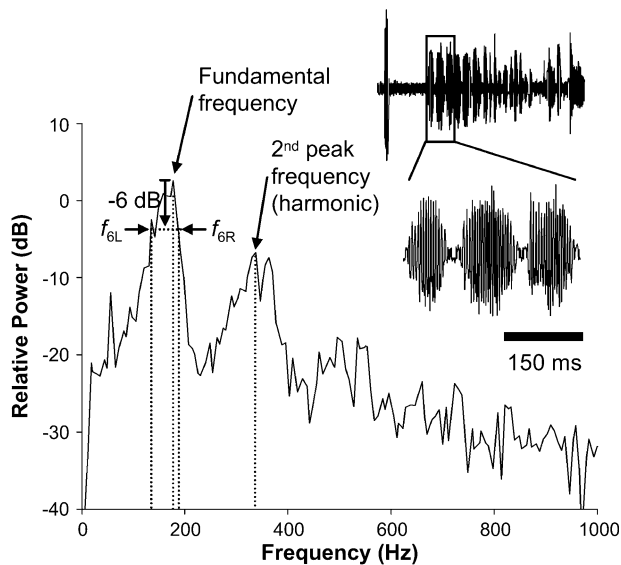
Figure 2
Power spectrum of the sound produced by a honeybee (*Apis mellifera*) immediately after a simulated attack, showing the acoustic parameters measured in this study. The fundamental frequency was defined as the peak with maximum energy (largest amplitude) in the spectrum while the peak with the next largest amplitude was designated the F2 frequency. To assess the bandwidth of the fundamental frequency, the left (f_{6L}) and right (f_{6R}) frequencies occurring 6 dB below the first peak in the spectrum were measured. The insets are oscillograms of the attack buzz (top) and the time-expanded section (bottom) used to generate the power spectrum.

Table 2
Sound characteristics emitted by each of our test species during flight

| Groups	Species	Observations	Fundamental frequency (Hz)	f_{6L} (Hz)	f_{6R} (Hz)	F2 frequency (Hz)	diffdB
Wasp	*Dolichovespula arenaria*	19	175.4 (2.2)	169.1 (2.3)	177.5 (5.8)	380.5 (4.5)	−1.9 (0.8)
	Vespula germanica	20	157.5 (3.0)	153.9 (2.1)	162.4 (3.1)	316.9 (5.8)	4.0 (0.7)
	Vespula vulgaris	7	169.3 (7.9)	166.7 (7.2)	178.4 (6.8)	344.5 (15.1)	1.52 (1.9)
Wasp mimic	*Spilomyia longicornis*	9	178.8 (15.0)	172.1 (16.0)	185.4 (15.2)	361.1 (30.8)	4.6 (2.1)
	Helophilus fasciatus	6	174.5 (6.8)	170.3 (7.5)	185 (7.3)	349.5 (13.3)	10.8 (2.1)
	Spilomyia sayi	7	174.8 (12.7)	168.3 (11.9)	180.5 (13.2)	349.5 (27)	3.4 (1.71)
Honeybee	*Apis mellifera*	25	171.2 (5.3)	167.5 (8.1)	175.7 (5.5)	339.5 (11.2)	5.6 (0.8)
Honeybee mimic	*Eristalis transversa*	5	216.7 (19.8)	207 (19.8)	223.1 (20.1)	431 (40.6)	6.2 (5.7)
	Eristalis arbustorum	15	218.7 (4.5)	214.7 (4.4)	223.9 (5.1)	431.9 (12.7)	−0.5 (1.7)
Bumblebee	*Bombus* sp.	5	140.7 (3.3)	135.7 (5.5)	143.5 (2.4)	280.4 (7.5)	−0.4 (1.8)
	Bombus impatiens	12	210.7 (2.4)	208.5 (2.5)	213.8 (2.5)	422.6 (4.7)	7.1 (1.12)
Bumblebee mimic	*Eristalis flavipes*	10	195.7 (6.8)	185.3 (5.4)	21.2 (7.0)	392.5 (14.5)	3.3 (1.5)
Nonmimetic flies	*Chrysops relictus*	7	159.4 (6.9)	156.2 (7.3)	162.6 (6.8)	319.1 (13.9)	10.4 (0.7)
	Sarcophaga spp.	21	171.3 (3.8)	165.6 (3.0)	169.5 (8.6)	342.4 (7.8)	4.17 (1.3)

Standard errors of the means are shown in parentheses. Note that the sample sizes differ between Tables 1 and 2 because not all specimens produced readily measurable sounds when engaged in the 2 activities.

their abdomen toward the forceps, indicating that they sting readily in response to simulated attacks. Although this readiness to sting resulted in lower quality condition of many of our recorded honeybees, it may well reflect natural circumstances, in which honeybees quickly lose their stingers when attacked by predators.

### Do wasp mimics sound like wasps on attack?

Overall, the results of the MANOVA on sounds (as characterized by PF and diffdB) made by the 3 wasp species (*D. arenaria*, *V. germanica*, and *V. vulgaris*) and 3 wasp mimic species *S. long-icornis*, *S. sayi*, and *H. fasciatus*) showed that there was significant variation among species in the sounds they made after attack (Wilks's lambda = 0.113, $F_{10,124}$ = 24.55, $P < 0.001$). Our post hoc Tukey tests revealed that each and every wasp species differed significantly from each and every wasp mimic species in fundamental frequency (all $P < 0.001$), with the 3 wasp species forming a statistically homogeneous subset and the 3 wasp mimics forming another. Likewise, the Tukey tests indicated that most wasp species differed significantly from the wasp mimic species in diffdB ($P < 0.05$)—the exception being *V. vulgaris* and *S. longicornis* ($P = 0.174$)—with wasps forming a subgroup which only partly overlapped with the
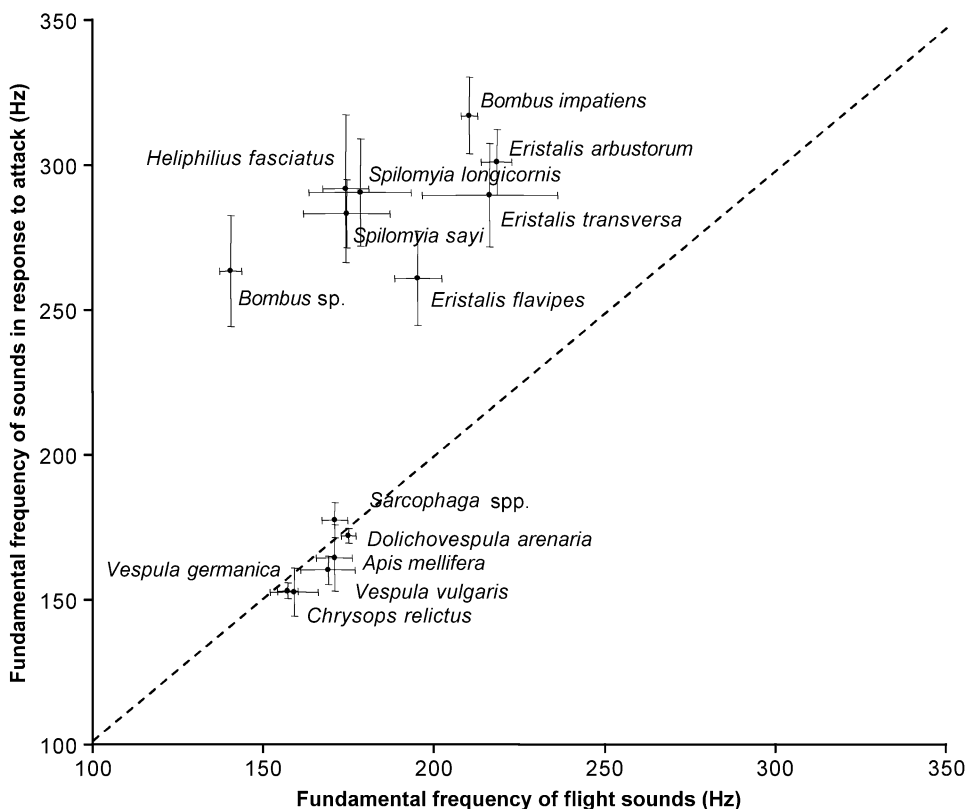
Figure 3
The fundamental frequencies of the sounds produced by tested insects after attack, compared with the sounds recorded during flight. All tested mimetic hoverflies and bumblebees responded with sounds that had a distinct higher fundamental frequency compared with the sounds produced during flight, immediately after attack. The buzzing sounds produced by nonmimetic flies, wasps, and honeybee *A. mellifera* showed little difference from their flight sound. Diagonal construct represents no change.
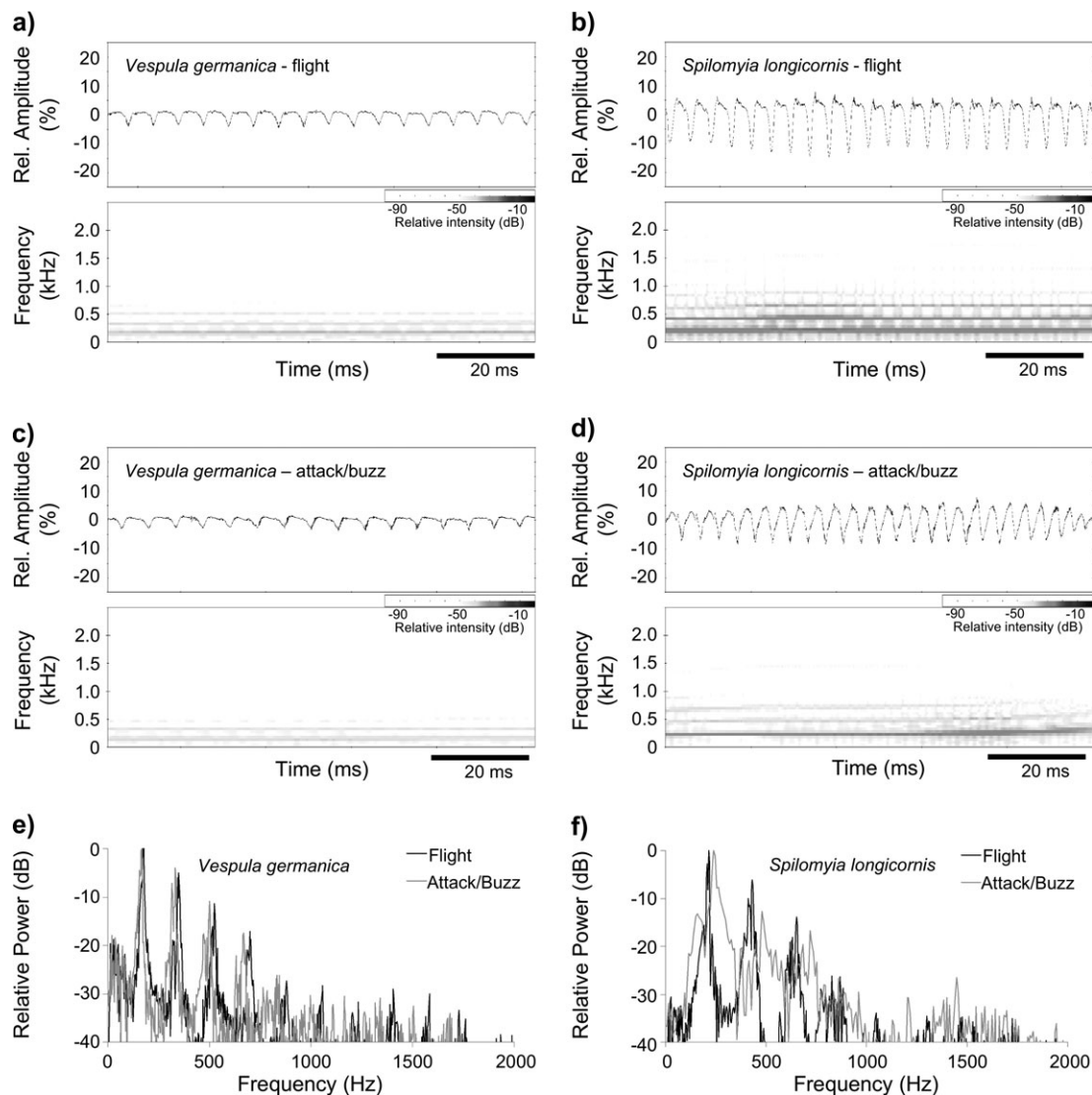
Figure 4
Temporal and spectral recordings of flight and buzzing sounds produced by the same individuals of wasp *Vespula germanica* (a, c, and e) and hoverfly *Spilomyia longicornis* (b, d, and f). Both flight (a and b) and buzzing sounds (c and d) are multiharmonic in structure but buzzing sounds show an increase in fundamental frequency and wider bandwidth (spectra in e and f, respectively).

wasp mimics. Results from our DFA confirm this general finding, in that each wasp mimic shows a similar Mahalanobis distance profile to other wasp mimic species tested and that the distances from wasps are relatively high (Figure 5). Likewise, the DFA classification probabilities indicated that although the sounds made by wasp-like hoverflies could often be confused with one another, there was little chance of them being misclassified as a wasp (mean probability of a wasp mimic specimen being classified as any of the 3 wasps was 0.0056, standard deviation [SD] 0.0125).

Do honeybee mimics sound like honeybees on attack?

Overall, the MANOVA results of the sounds made by honeybee (*A. mellifera*) and the 2 honeybee mimic species (*E. transversa* and *E. arbustorum*) were indicative of significant among-species differences in the sounds produced after the simulated attacks (Wilks's lambda $= 0.254$, $F_{4,68} = 16.76$, $P < 0.001$). Tukey tests confirmed that the sounds produced by the honeybee differed significantly from both honeybee mimic species in fundamental frequency (all $P < 0.001$). Once again,

the honeybee formed a statistically distinct subset, and the honeybee mimics formed another homogeneous subset. However, differences in diffdBs between honeybee mimic hoverflies and their honeybee models were not statistically detectable, and only the 2 honeybee mimics differed significantly in diffdB ($P = 0.03$). Our DFA supports this general finding, with the 2 honeybee mimics showing almost identical similarity profiles, which differ markedly from *A. mellifera* (Figure 5). The DFA classification probabilities indicated that the sounds made by honeybee mimics could occasionally be confused with one another (mean misclassification rate 0.122, SD 0.118), but there was little chance of the mimics being misclassified as a honeybee on the basis of their sounds (mean classification as a honeybee 0.012, SD 0.041).

Do bumblebee mimics sound like bumblebees on attack?

The results of the MANOVA indicate that the 2 species of bumblebee and their hoverfly mimic do not statistically differ in the sounds they produce immediately after attack (Wilks's
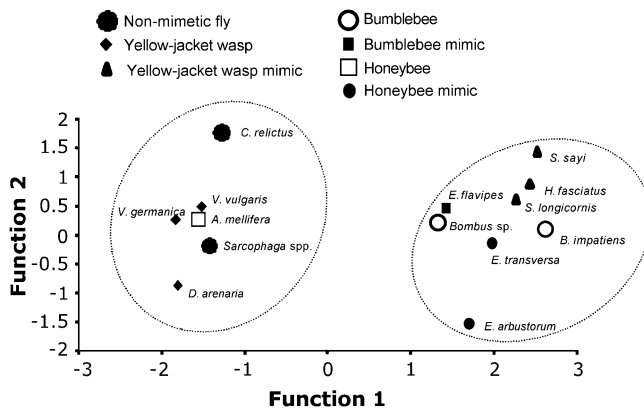
Figure 5
DFA of sounds produced after attack. The points indicate the centroid of each measured species. The left ring represents the wasps, nonmimetic flies, and honeybee, whereas the right ring represents the mimetic hoverflies and bumblebee.

lambda = 0.716, $F_{4,44} = 2.00$, $P = 0.111$). Both models and the mimic were categorized under the same homogenous subset, for both dependent variables although *B. impatiens* and *Bombus* sp. differed significantly in their fundamental frequency ($P = 0.029$). Our discriminant analysis further supports the general conclusion that the 2 bumble bee species, overall, share some similarities in the sound they produce on attack, with their bumblebee mimic, *E. flavipes* (Figure 5). Indeed, the DFA overall mean misclassification probability of *E. flavipes* as one or the other species of bumblebee was 0.235, SD 0.129.

One might wonder whether it is only the bumblebee mimic *E. flavipes* that sounds like bumblebees. If we include all species of hoverfly and the 2 species of bumblebee in the MANOVA, then there is a significant species effect (Wilks's lambda = 0.481, $F_{14,124} = 3.92$, $P < 0.001$). However, Tukey post hoc tests revealed that no pair of species (drawn from bumblebees and hoverflies) differed significantly ($P > 0.05$) in fundamental frequency. For diffdB, none of the 6 hoverfly species differed significantly from *Bombus* sp. (all $P > 0.05$) and only one hoverfly (*E. arbustorum*) differed significantly from *B. impatiens* in diffdB ($P = 0.007$) as well as a range of other hoverflies (see below). Collectively, this indicates that although *E. flavipes* resembles the sound of a bumblebee, it does not resemble bumblebees any better or worse than do other species of hoverfly.

Do hoverfly mimics sound like one another, independent of the model they resemble?

Multivariate analysis of the sounds made by all 6 species of mimetic hoverfly on attack revealed that the species differ significantly in overall sounds produced in response to simulated attacks (Wilks's lambda = 0.468, $F_{10,96} = 4.43$, $P < 0.001$). Our post hoc analyses, however, confirmed that the fundamental frequency did not differ significantly among species (all $P > 0.05$). Therefore, the primary driver of the significant effect was diffdB, and here the honeybee mimic *E. arbustorum* differed significantly from *E. flavipes, S. sayi, S. longicornis,* and *H. fasciatus* (all $P \leq 0.007$). Separate univariate analyses of variance for all 5 sound variables indicated that only diffdB differs significantly among hoverfly species ($P < 0.001$). Collectively, this indicates that the bee mimic *E. arbustorum* was relatively distant from the rest of the hoverflies in diffdB as well as its assumed honeybee model (Figure 5). Our discriminant analysis supports the conclusion, in that wasp mimics

sounded similar to bee mimics based on Mahalanobis distance, both of which differ from the wasp and honeybee models as well as the nonmimetic flies. Thus, overall, hoverfly mimics tend to sound more like other species in the same taxonomic group when attacked, independent of the model they morphologically resemble.

DISCUSSION

Numerous studies have demonstrated that sound can be used as part of a defensive response of animals (e.g., Haskell 1961; Lane and Rothschild 1965; Rothschild and Haskell 1966; Rowe and Owings 1978; Masters 1979; Rowe et al. 1986; Dunning and Krüger 1995; Hristov and Conner 2005; Barber and Conner 2007; Hill 2007). "Startle displays," which are defined as a sudden conspicuous change in appearance and behavior of an animal (see Stevens 2005), include "startle sounds" that can be used by some insects to deter predation by potential predators (Vallin et al. 2005; Hill 2007). Sounds may also function as a signal of unprofitability to would-be predators (Masters 1979). For example, Hristov and Conner (2005) have shown that unpalatable tiger moths (Arctiidae) produce a sound that serves to deter predation by bats. Similarly, in a recent study, it has been shown that the silk moth caterpillar, *Antheraea polyphemus* (Saturniidae), produces "clicking" sounds using mouth parts to advertise its secondary line of defense, regurgitating an unpalatable fluid (Brown et al. 2007). In some of these cases, acoustic signals may enhance protection by exploiting multiple sensory channels of communication to the potential predator (multimodal signaling) (Rowe and Guilford 1999).

To our knowledge, this is the first study to experimentally investigate the sounds produced by mimetic hoverflies and their presumed models (bees and wasps) on attack in the context of Batesian mimicry. We predicted that hoverflies would acoustically mimic the hymenopteran models they morphologically resemble more closely than related hoverflies that resemble a different model. We failed to detect any such trend among wasp mimics and models or between honeybee mimics and their honeybee model. Hoverflies, which resemble a number of different Hymenoptera, generally sound much more like one another (with one exception in our case, *E. arbustorum*) than the models they resemble when they were artificially attacked. However, ruling out acoustic mimicry entirely is extremely challenging because Batesian mimicry does not require perfect resemblance, only sufficient resemblance to the mimic's model to confer a selective advantage. Moreover, we are implicitly making assumptions about the "ear of the beholder," namely, that the sound differences detectable by the recording equipment are also detectable by any predators.

We note that one species of bumblebee mimic (*E. flavipes*) did indeed share many of the acoustic attributes with their proposed models, so it is particularly hard to rule out acoustic mimicry in this case. However, the fact that *E. flavipes* is no closer of an acoustic mimic to its visual model than are other hoverfly species (Figure 5) suggests that either this similarity has arisen by chance or that all the hoverfly species sampled are bumblebee mimics.

Contrary to the suggestion made by Waldbauer (1988), we found no evidence that our wasp mimic hoverflies sounded like wasps when grabbed. Perhaps Waldbauer (1988) had the sound of a generic hymenopteran in mind, but of course, we cannot reject the possibility of acoustic mimicry in other, as yet untested, species pairs. In fact, the 2-winged Diptera and 4-winged Hymenoptera are known to have differences in their mechanical properties of their flights (Dudley 2000). Therefore, it is possible that morphological constraints prevent

visual mimics from adapting similar sound characteristics of their models on attack. It is also possible that hoverflies are only (at very best) Batesian acoustic mimics in a general sense, producing sounds which evoke a general hymenopteran, regardless of the model that they actually resemble. Future studies could investigate this possibility by comparing the sound produced by wasp and bee mimic hoverflies to a range of model and nonmodel species of Hymenoptera.

Although birds are highly visual predators, acoustic signals in mimetic hoverflies could be used as part of a defensive response against predators when visual signals, per se, fail to protect the potential prey. All the mimetic hoverflies and bumblebees tested in our experiments exhibited a high-frequency sound when attacked. By contrast, all our wasp and nonmimetic fly species simply tried to fly away when they were artificially attacked and did not produce a distinct buzz. A basic and tentative rule is that if a species can actively defend itself after capture or if it can readily avoid being captured, then postcapture signaling does not evolve. Nevertheless, comparative analyses of the sounds made by many more species is necessary before the validity of such rules can be properly evaluated.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco. oxfordjournals.org/

FUNDING

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