Behav Ecol Sociobiol (2010) 64:1485–1494
DOI 10.1007/s00265-010-0963-7

ORIGINAL PAPER

Risk trading in mating behavior: forgoing anti-predator responses reduces the likelihood of missing terminal mating opportunities

Marie Lafaille · Gaëlle Bimbard · Michael D. Greenfield

Received: 26 January 2010 / Revised: 22 March 2010 / Accepted: 14 April 2010 / Published online: 28 April 2010
© Springer-Verlag 2010

Abstract Life history theory predicts that organisms make certain adjustments to their current and future reproductive effort such that fitness is maximized. Moreover, these adjustments may be fine tuned in response to risks of attack by natural enemies. Thus, we may predict that as an organism ages it will accept increasing levels of exposure to predators during mating activities, effectively trading the risk of losing terminal mating opportunities for the risk of predation. We tested this prediction in an acoustic moth, *Achroia grisella*, in which females orient toward and evaluate males based on their ultrasonic calling song, and both sexes may be vulnerable to predation by insectivorous bats while in flight as well as on the substrate. In the latter situation, singing males and orienting females show silence and arrestment responses, respectively, when presented with synthetic bat echolocation signals broadcast above a threshold amplitude. We found that both males and females become less sensitive to these broadcasts over the course of their brief reproductive periods, 7 and 5 days, respectively. Over the same periods, sensitivity to male song in both males and females remains constant, and relatively little senescence in sexual behavior is observed. These results support the risk trading hypothesis, and they indicate that life history principles may apply over a very short lifespan.

Keywords Acoustic communication · Life history trade-off · Phonotaxis · Reproductive value · Sexual selection · Terminal investment

Communicated by N. Wedell

M. Lafaille · G. Bimbard · M. D. Greenfield (✉)
Institut de Recherche sur la Biologie de l'Insecte,
CNRS UMR 6035, Université François Rabelais de Tours,
37200 Tours, France
e-mail: michael.greenfield@univ-tours.fr

Introduction

Life history theory predicts that organisms adjust reproductive effort over their lifespan such that fitness is maximized (Roff 1992; Stearns 1992). Thus, we may anticipate that relatively young individuals, with high expectations of survival, will devote less effort to current reproduction and thereby reserve much energy for future opportunities—given the constraint that energy allocated now will necessarily reduce that which can be spent later. Conversely, older individuals, having little expectation of survival regardless of their current allocation, may reserve a relatively small proportion of their available energy for future reproduction (Clutton-Brock 1984). This basic prediction has been confirmed in numerous cases (e.g., Ericsson et al. 2001; Descamps et al. 2007; Bercovitch et al. 2009; Isaac and Johnson 2005), including insects with short reproductive lifespan (Javoiš and Tammaru 2004). In general, organisms are observed to modify their reproductive investment in accordance with various intrinsic (indications of chronological or physiological age) or extrinsic (indications of resource availability in the environment) factors. The modifications typically studied have been tangible components of investment, such as the production of eggs (e.g., see Fletcher et al. 1994; Sirot et al. 1997).

Time may also constrain an organism's sexual activities independently of energy by simply limiting opportunities for mating and reproduction (Real 1990). Here, we might expect a young individual, one that expects future mating opportunities, to be more discriminating in the choice of a partner than an older individual who had not yet mated, or at least had not mated recently—provided that time spent on a given mating limits opportunities for other matings at that general time. In this latter case of older individuals, it may be preferable to mate with any available partner than to risk not mating at all because an acceptance threshold remains too high. The risk of attack by natural enemies during sexual activities can modify further the way in which an organism adjusts its behavior. Sexual activities, including advertisement signaling, searching for and evaluating mates, and courtship, are notoriously conspicuous and often attract the attention of predators and parasites (Zuk and Kolluru 1998). Thus, animals often reduce these activities when natural enemies, or indirect indications of the presence of enemies, are perceived (e.g., Hedrick and Dill 1993; Fuller and Berglund 1996; Koga et al. 1998).

The two above risks may then be combined in a life history context, which leads to the prediction that an animal should respond to natural enemies more strongly when many future reproductive opportunities are expected (Candolin 1998), as when relatively young. That is, an older individual may raise its threshold for responding to predators and parasites during sexual activities, effectively ignoring the second risk in order to avoid the first—the possibility of missing its terminal mating opportunities. Such adjustments have been observed in the black goby, where 4–5-year-old males will spawn in the presence of predator fish species whereas 2–3-year-old males normally do not (Magnhagen 1990), but in general they have not been studied in depth. In particular, we do not know the precise schedules with which sexual and anti-predator activities are regulated—and coordinated—over an animal's lifespan, whether these schedules reflect primarily chronological age or a variety of physiological factors, and whether these life history principles operate regardless of the organism's absolute lifespan.

We addressed the question of how animals might adjust their responses to natural enemies in order to avail themselves of terminal mating opportunities by studying the sexual activities of an acoustic moth, *Achroia grisella* (Lepidoptera: Pyralidae). Male *A. grisella* broadcast an ultrasonic advertisement song by wing-fanning while remaining stationary on the substrate (Spangler et al. 1984). The song consists of a rhythmic series of paired pulses of high-frequency (70–130 kHz) sound that a male delivers more or less uninterruptedly for 6–10 h during the night. Females orient toward singing males up to 1 m distant, and they generally do so by running, rather than flying. Males also respond to surrounding males, which they do by temporarily accelerating the pulse-pair rhythm in their song (Jia et al. 2001). Female *A. grisella* prefer male songs that have faster pulse-pair rates (Jang and Greenfield 1996), and this acceleration response may represent a form of signal competition between neighboring males.

Both male and female *A. grisella* adults have atrophied mouthparts (the coiled proboscis is unusually short), and they consequently neither feed nor drink (Greenfield and Coffelt 1983). Thus, adults depend entirely on resources accumulated during the larval stage for their energy reserves, which is probably responsible for their brief lifespan: In the laboratory, males typically survive 7–14 days following the adult molt, while females survive several days less. Males begin singing and can mate within 1 h of the adult molt, and they continue their nightly singing routine until shortly before death (Brandt and Greenfield 2004). Females too become sexually receptive shortly after the adult molt, and their receptivity lasts for 5–7 days (Brandt et al. 2005). Males can mate approximately every 24 h, whereas females normally mate only once and become permanently refractory thereafter. Previous studies have shown that certain characters of a male's song (Brandt and Greenfield 2004) and a female's response to male song (Brandt et al. 2005) do not change appreciably over their reproductive periods. For example, in male song the mean pulse-pair rhythm only slowed 3.6% as individuals aged from 1 to 4 days, and another 5.6% as they aged from 4 to 7 days (Jang et al. 1997). Similarly, the mean peak amplitude of male song remained constant between ages 1 and 4 days, and then only declined 6.7% between ages 4 and 7 days.

In addition to responding to male song, both male and female *A. grisella* exhibit specialized responses to synthetic ultrasonic broadcasts that represent the echolocations of insectivorous bats. As in many nocturnal Lepidoptera (especially Pyraloidea, Geometroidea, and Noctuoidea; Waters 2003), *A. grisella* in flight often cease wing beating and plunge to the ground when they perceive these synthetic signals (Rodriguez and Greenfield 2004). In addition to this well-known aerial response, *A. grisella* engaged in sexual activities while on the substrate, including mate calling and searching, also exhibit specific behavior when presented with synthetic bat echolocations. Singing males generally become silent (Greenfield and Baker 2003), and females orienting toward a singing male may arrest movement (Greenfield and Weber 2000; Greig and Greenfield 2004), presumably to eliminate the production of inadvertent sounds generated by running (cf. Acharya and McNeil 1998; Jones et al. 2002 for comparable behavior in other Lepidoptera). These two responses may reduce vulnerability to attacks from bat species that passively localize non-flying insect prey by listening to their sounds and then glean them from the substrate (Neuweiler 2000; Arlettaz et al. 2001). Previous studies of *A. grisella* have revealed the specific characters of these synthetic echolocation signals that elicit silence and arrestment responses, and how these signals are distinguished from male song. Findings indicating that these specific characters largely coincide with the features of actual 'searching phase' echolocations of various species of gleaning bats argue that the responses are adaptations to avoid attacks from these predators (Greenfield and Hohendorf 2009).

To determine whether *A. grisella* maximize terminal mating opportunities by reducing anti-predator behavior, we studied the way in which males and females modulated their silence and arrestment responses over the course of their reproductive lifespans. To achieve a quantitative determination, we measured the threshold amplitude levels of synthetic echolocation signals that elicited these responses and asked whether they increased with age. Such increases may imply that individuals accept the risk of bat predation as their expected reproductive lifespan nears its end, but they may also result simply from physiological aging, i.e., senescence (Bonduriansky and Brassil 2002): Both mechanical (tympanal membranes) and nervous (peripheral neurons investing the tympanum) components of ears could function less effectively in older individuals, and threshold amplitude levels might rise as a result. To distinguish between senescence and risk trading, we also measured the threshold amplitude levels of male song that elicited sexual and competitive responses in females and males, respectively. Because the same mechanical and peripheral neuronal apparatus are used in perceiving both predators and conspecifics, the latter either as potential mates or rivals, finding that thresholds increase with age in response to predator signals but not to conspecific song would support an active risk-trading process. On the other hand, response thresholds that increase with age in both contexts would favor the senescence explanation, and indicate as well that older females become more, not less, discriminating when evaluating potential mating partners. Thus, females might actually suffer a loss of terminal mating opportunities despite their concurrent trend to ignore predators.

Materials and methods

Population studied and rearing

We studied insects from an *A. grisella* population derived from several hundred larvae collected at abandoned honeybee colonies near Baton Rouge, Louisiana in August 2006. The population was bred in the laboratory with a protocol that reduced the loss of genetic variation. We reared the insects on a synthetic diet (Jang and Greenfield 1996) and maintained them under a 12:12 L:D photoperiod and 25±1°C. Under these conditions, generations lasted approximately 45 days.

General experimental methods

All experiments were conducted on unmated adults to ensure a standard physiological state. We isolated pupae from the rearing boxes and kept them individually in 30-ml containers, where they eclosed to adults. We tested the responses of female and male adults to synthetic bat echolocations and male song at regular intervals following the adult molt: 1, 3, and 5 days for females and 1, 4, and 7 days for males, who normally survive longer. Tests were conducted within an acoustically insulated chamber maintained at 25°C and illuminated by diffuse red light from a 25-W incandescent bulb during the experiments. We placed the insects within the acoustic chamber 30 min prior to testing to allow for acclimatization.

Our basic approach was to conduct a series of playback trials that determined the threshold amplitude of synthetic bat echolocations and male song that elicited anti-predator and sexual responses, respectively. Thus, we created standard acoustic signals to represent bat predators and male *A. grisella*, and we presented them to each test insect in an ascending series of incremented amplitude levels until a response was observed or the highest level was reached. We began our presentations at a level equivalent to or below the minimum expected threshold value, and we waited a minimum of 30 min between successive trials to avoid potential habituation to the signals. To that end, we also presented the signals for relatively short durations, and we used the minimum number of increments that would span the range of expected threshold values but still provide somewhat precise information on a given insect's threshold. In testing females, we returned the insects to their individual cups following each trial and kept them within an acoustically insulated box until the next trial. Males were simply protected by a barrier of acoustic foam that insulated them from test signals, and from other test males, during the intervals between their successive trials. All tests were conducted during the first 6 h of the night, the natural activity period of adult *A. grisella*, and of foraging bats. With the exception of experiment 2, we used the method of presenting an ascending sequence of amplitude levels to determine thresholds rather than employing a randomized block design in order to reduce the number of trials necessary for most individuals. Many individuals responded by the third or fourth amplitude level, and we were thus afforded the time for sampling more individuals in each part of the study (Greenfield and Hohendorf 2009). Tests of insects of the same age were not performed synchronously, which precluded the possibility that an environmental factor was confounded with age.

We created synthetic bat echolocation signals and male song stimuli via standard digital procedures and broadcast these signals to the test insects in a circular arena mounted on a turntable or in a rectangular screen tunnel. Previous tests had shown that the screen used did not attenuate or otherwise distort the broadcasts. Synthetic bat echolocation signals were created with a signal editing program (Cool-Edit verson 1.53; Syntrillium; Phoenix, AZ, USA) that generated a 2.5-ms pulse of sine waves whose frequency descended linearly from 100 to 25 kHz over its duration. This pulse was copied and edited digitally (BatSound Pro

4.0; Petterson Elektronik AB; Uppsala, Sweden) to produce a train of identical pulses repeated at a rhythm of 15 s^{-1}. These spectral and temporal features are representative of the searching phase echolocations of gleaning bat species that would be likely to menace insects moving and singing on the substrate, including *A. grisella* (Greenfield and Hohendorf 2009). To create male song stimuli, we began with a digitized recording of a male from our Louisiana population. This recording was made with a condenser ultrasound microphone (model CM16/CMPA; Avisoft Bioacoustics; Berlin, Germany; frequency response ±3 dB, 20–150 kHz) whose output was digitized with an analog/digital converter (model UltraSoundGate 416–200; Avisoft Bioacoustics) at 16 bits and 500,000 samples s^{-1}. From this recording, we selected a single 100-μs pulse with an envelope and spectral properties that were typical of the population. Using BatSound Pro 4.0, we copied this pulse to create a train of pulse pairs (two pulses whose onsets were separated by 500 μs) repeated at 90 s^{-1}, the average pulse-pair rhythm observed at 25°C in the population. Both the synthetic bat echolocation signal and the male song stimulus were saved as digital files.

During a playback trial, we continuously looped the digital file of the synthetic bat echolocation signal or the male song stimulus on a personal computer, converted the digital signal to analog with an input/output card (DAQcard 6062E; National Instruments; Austin, TX, USA), and sent the analog signals at 214,285 samples·s^{-1} to a loudspeaker (model ScanSpeak; Avisoft Bioacoustics; frequency response ±2 dB, 60–110 kHz) that was amplified by a power amplifier (Avisoft Bioacoustics). In all experiments, we adjusted the peak amplitude, as presented at the location of the female or male in the arena, of the broadcast signals with the aid of a sound pressure level (SPL) meter (model 1982; General Radio; Concord, MA, USA; flat frequency response from 30 to 20,000 Hz), confirmed with a calibrator (model 1987; General Radio). We implemented the method of peak equivalents (pe) by relating the millivolt output of a continuous 16-kHz broadcast, as measured by the condenser ultrasound microphone, to the SPL of this broadcast, as registered by the SPL meter. We then noted the millivolt output of the synthetic bat echolocation or song stimulus broadcast as measured by the microphone, and we adjusted the gain on the loudspeaker amplifier until this millivolt output was equivalent to the desired dB peSPL (0 dB= 20 μPa; Jang and Greenfield 1996).

Experiment 1: how does the female arrestment response to synthetic bat echolocation signals change over the adult lifespan?

We studied the responses of females to synthetic bat echolocation signals presented while they were orienting toward male song (Greenfield and Weber 2000). Tests were conducted in a screened tunnel (42×12×5 cm) that essentially constrained the movement of the insect along one dimension, thereby allowing us to control the amplitude of the signal to which the insect was exposed. Because most of the energy broadcast from the high-frequency loudspeakers that we used was concentrated along the central axis, we would not have been able to ensure the presentation of a given signal amplitude were the insects free to move within a wide arena. We released a test female at one end of the tunnel and then began the broadcast of the male song from a loudspeaker placed at the opposite end of the tunnel and directed at the female. The male song stimulus was adjusted to a standard 75 dB SPL, equivalent to an average male at 15 cm, and it was continued for 30 s. Females normally began running toward the song stimulus within several s of its onset. When the female had moved 2 cm toward the song stimulus, we presented a 1-s broadcast of the synthetic bat echolocation signal from a second loudspeaker placed at the opposite end of the tunnel, behind the release point, also directed at the female. We adjusted the amplitude of the echolocation signal to 86, 92, and 98 dB peSPL in the three successive playback trials in which a given female might be tested. These amplitude levels were selected to represent a ‘typical’ gleaning bat at 2, 1, and 0.5 m, respectively (see Waters and Jones 1995; Brinklov et al. 2009 on amplitude measurements of gleaning bats in the field). Once a female responded to a particular amplitude level, we conducted an additional trial in which we repeated the presentation of the level one increment below to confirm that the observed response was given at her threshold and not at a higher level. For those females who responded in the first trial, the playback of the echolocation signal at 86 dB, the additional trial was conducted with a playback at 80 dB.

We tested 19 females repeatedly at each of three ages: 1, 3, and 5 days past the adult molt. We designated a response to the synthetic bat echolocation signal as arrestment of movement within 3 s of the onset of the signal. We noted the amplitude of the echolocation signal at which the arrestment response was observed, latency of the response as measured from signal onset, and whether the female resumed movement toward the speaker broadcasting the male song stimulus during its 30-s duration.

Experiment 2: how does the male silence response to synthetic bat echolocation signals change over the adult lifespan?

We studied the responses of males to synthetic bat echolocation signals while they were singing (Greenfield and Baker 2003). Tests were conducted on males held in small screen cages (1.5 cm diam., 3 cm height) that were spaced at regular intervals along the circumference of the circular arena. The loudspeaker broadcasting the echolocation signals was placed at one side of the arena. We rotated the arena to position a given test male adjacent to the loudspeaker, 15 cm distant, and conduct a playback trial. Each male cage was protected by a shield of acoustic insulation foam on all sides except that facing the loudspeaker. Thus, males were not exposed to the songs of neighbors, and they were only exposed to playbacks during their own trials.

We began each trial by monitoring the test male's song for 3 min with the condenser ultrasound microphone and with a bat detector (model D230; Petterson Elektronik AB) to ensure that he was singing continuously. Males that interrupted their song spontaneously during this phase were not tested further. For males that did sing without such interruptions, we then presented a 1-s broadcast of the synthetic echolocation signal and used the condenser microphone to record his song for 1 min prior to the playback, during the playback, and for 2 min following the playback. This recording captured both the male's song and the playback, and it was later analyzed with BatSound Pro 4.0. In this analysis, we designated a silence response when a male who otherwise sang uninterruptedly missed at least one pair of pulses during the 1-s playback. We noted the echolocation signal amplitude that elicited a silence response as well as the total duration of the silence.

We tested three groups (n=13, 12, 14) of males aged 1, 4, and 7 days, respectively. Each male was tested with a randomized series of six echolocation signal amplitudes: 67, 73, 79, 85, 91, and 97 dB peSPL.

In another series of playback trials, we examined how the latency of the male's silence response changed over his lifespan. Here, we also tested three groups (n=28) of males aged 1, 4, and 7 days, respectively, but presented each with a standard 85 dB playback only. We noted whether a silence response was observed at this echolocation signal amplitude and measured its latency, defined as the interval from signal onset to the first missing pair of pulses in the male's song. Unlike the other anti-predator and sexual responses evaluated in this study, the male silence response varies in latency, and we judged that standard measures of this feature would provide additional information on the level of response.

Experiment 3: how does the female sexual response to male song vary over the adult lifespan?

We determined the threshold amplitude levels of male song that elicited phonotaxis in females (Greenfield and Hohendorf 2009). Tests were conducted with the same rectangular screen tunnel and other apparatus described in experiment 1 except that only one loudspeaker, broadcasting the male song stimulus, was used. As before, a female was released at the end of the tunnel opposite the loudspeaker, and the 30-s song broadcast began when she had remained in place for 10 s. We tested each female with an ascending sequence of five song stimulus amplitudes: 63, 69, 75, 81, and 87 dB peSPL. As in experiment 1, once a female responded to a particular amplitude level, we conducted an additional trial in which we repeated the presentation of the level one increment below. For those females who responded in the first trial, the playback of the song stimulus at 63 dB, the additional trial was conducted with a playback at 57 dB.

We tested 31 females repeatedly at each of three ages: 1, 3, and 5 days past the adult molt. We designated phonotaxis as movement to within 5 cm of the loudspeaker and remaining in that zone for a minimum 5 s during the broadcast.

Experiment 4: how does the male competitive response to male song vary over the adult lifespan?

We determined the threshold amplitude levels of male song that elicited a competitive response, i.e., an elevated song rhythm, in males (Jia et al. 2001). Tests were conducted with the same circular arena and other apparatus described in experiment 2 except that the loudspeaker broadcast the male song stimulus rather than synthetic bat echolocation signals. As before, the arena was rotated so that a given caged male was positioned adjacent to a loudspeaker and his singing was monitored for 1 min with the ultrasonic condenser microphone and the bat detector. For males that sang uninterruptedly during this phase, we then began a 2-min broadcast of the song stimulus and continued recording the male's song with the condenser microphone. We later analyzed the recording with BatSound Pro 4.0, measuring the pulse-pair rate of the male's song during five randomly selected 1-s intervals prior to playback and during five randomly selected 1-s intervals during the playback. A competitive response was designated as an increase of 3% or more in the mean pulse-pair rate observed during the playback intervals compared with the pre-playback intervals.

We tested three groups (n=14, 14, 17) of males aged 1, 4, and 7 days, respectively. Each male was tested with an ascending series of three song stimulus amplitudes: 66, 72, and 78 dB peSPL.

Results

Experiment 1: how does the female arrestment response to synthetic bat echolocation signals change over the adult lifespan?

We found that the threshold amplitude eliciting the arrestment response in females increased significantly over the three test ages (Fig. 1a). Unlike the other three experiments, experiment 1 required that the test insect perform two separate, successive activities: movement toward the male song stimulus and then arrestment of this movement in response to the synthetic bat echolocation signal. Most of the tested females (20/23) oriented toward the song stimulus when 1 day old, as well as at the two subsequent test ages, and all but one of these 20 females exhibited an arrestment response during the ascending series of signal amplitudes presented at each of the three ages. One female never responded at age 5 days.

The distributions of threshold amplitude levels of the synthetic echolocation signal that elicited an arrestment response in female *A. grisella* differed significantly between the three age classes (1 vs. 3 days—McNemar test (two-tailed), χ^2=10.0, *df*=3, *p*=0.019; 3 vs. 5 days—χ^2=9.33, *df*=3, *p*=0.025; 1 vs. 5 days—χ^2=16.0, *df*=3, *p*=0.001; see Zar 1999, p. 171). For all comparisons, $p<0.05$ following correction for multiple tests by the sequential Bonferroni procedure (Holm 1979). Whereas all females showed threshold amplitudes of 86 or 92 dB peSPL when 1 day old, and all but one female showed an arrestment response by the 92 dB level when 3 days old, 40% of the females did not respond until presented with the 98 dB level when 5 days old.

When females exhibited an arrestment response to the synthetic echolocation signal, a certain percentage of them resumed moving toward the male song stimulus shortly thereafter. This resumption of orientation within 30 s was more common in females when 5 days old than when 1 day old [McNemar test (two-tailed), χ^2=4.9, *df*=1, *p*=0.027], but significant differences were not observed for the other two age comparisons (1 vs. 3 days old—χ^2=1.13, *df*=1, *p*=0.29; 3 vs. 5 days old— χ^2=1.5, *df*=1, *p*=0.22).

The duration of the arrestment response following the brief synthetic echolocation signal also differed significantly between the three age classes. Females resumed orienting sooner when they were 3 days old than when 1 day old

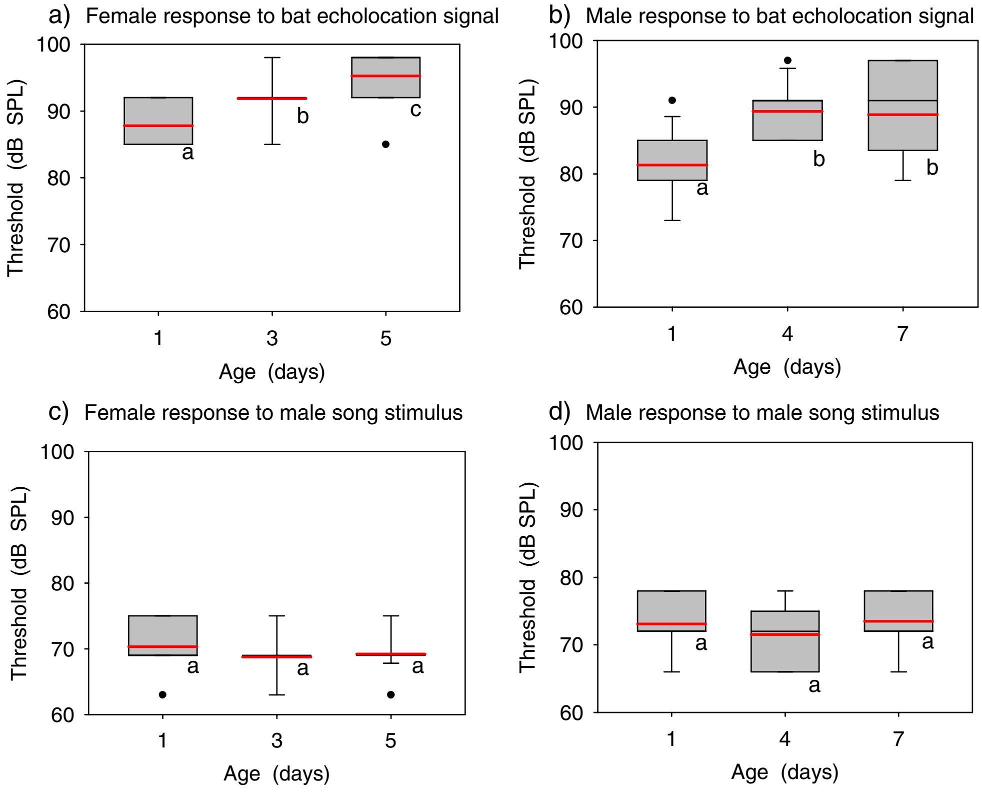

Fig. 1 Response thresholds as a function of adult age for anti-predator and sexual behavior in female and male *A. grisella*. Box plots indicate mean (*solid red line*), median (*solid black line*), 25–75% range (*box*), 10–90% range (*whiskers*), and outliers; *missing boxes* imply that 75% or more of the values equaled the median. **a** Amplitude of synthetic bat echolocation signal eliciting an arrestment response in females orienting toward male song. *Boxes followed by the same letter* indicate values that are not significantly different (McNemar test, two-tailed; α=0.05). **b** Amplitude of synthetic bat echolocation signal eliciting a silence response in singing males. *Boxes followed by the same letter* indicate values that are not significantly different (Kruskal–Wallis test, with Dunn's method for multiple pairwise comparisons, α=0.05). **c** Amplitude of male song stimulus eliciting orientation in females. *Boxes followed by the same letter* indicate values that are not significantly different (McNemar test, two-tailed; α=0.05). **d** Amplitude of male song stimulus eliciting a competitive response, an increment in pulse-pair rate, in singing males. *Boxes followed by the same letter* indicate values that are not significantly different (*G* test, α=0.05)

[Wilcoxon signed-rank test (two-tailed), $n=19$, $z=-2.13$, $p=0.034$], and when 5 days old, females resumed orienting sooner than when either 3 days or 1 day old (3 vs. 5 days old—$n=19$, $z=-2.71$, $p=0.007$; 1 vs. 5 days old—$n=19$, $z=-3.01$, $p=0.003$). For all comparisons, $p<0.05$ following correction for multiple tests by the sequential Bonferroni procedure (Holm 1979).

We observed no significant differences between the latency of the female arrestment response, measured from the onset of the synthetic echolocation signal, exhibited by the three age classes (Friedman test, $\chi_r^2=0.105$, $df=2$, $p=0.95$). Median latencies were 1.44, 1.27, and 1.27 s for females when 1, 3, and 5 days old, respectively.

Experiment 2: how does the male silence response to synthetic bat echolocation signals change over the adult lifespan?

As in the female arrestment response to synthetic bat echolocation signals tested in experiment 1, we found that threshold amplitudes for the male silence response to these signals also increased with age (Fig. 1b). Among males in the three age classes tested, only one individual, in the 4-day-old age class, did not exhibit a silence response during the ascending series of signal amplitudes presented. Overall, the distributions of threshold amplitudes differed significantly among the three age classes (Kruskal–Wallis test, $H=10.34$, $df=2$, $p<0.01$). A multiple comparisons analysis (Dunn's method) revealed a significant difference between 1- and 4-day-old males ($Q=12.46$, $p<0.05$), as well as between 1- and 7-day-old males ($Q=11.13$, $p<0.05$), but not between 4- and 7-day-old males ($Q=0.30$, $p>0.05$).

Our test in which we presented a single amplitude level (85 dB peSPL) of the synthetic echolocation signal showed that the proportion of males exhibiting a silence response decreased with age (Fig. 2a) while the latency of their response lengthened (Kruskal–Wallis test, $H=6.49$, $df=2$, $p<0.05$; Fig. 2b). Moreover, 96.4% of 1-day-old males, 67.9% of 4-day-old males, and 28.6% of 7-day-old males exhibited a silence response (1 vs. 4 days—test of two binomial proportions, $z=3.01$, $n=28$, $p=0.003$; 4 vs. 7 days—$z=3.20$, $p=0.001$). For both comparisons, $p<0.05$ following correction for multiple tests by the sequential Bonferroni procedure (Holm 1979). For all individuals designated as responding except three 1-day-old males, the length of the response exceeded 0.5 s, the equivalent of missing approximately 25 pulse pairs of song. The median latency of the silence response increased from 154 ms in 1-day-old males to 314 ms in 7-day-old males (Dunn's method for multiple comparisons, $Q=2.50$, $p<0.05$). On the other hand, we found no influence of age on the duration of the silence response (Kaplan–Meier log-rank survival analysis, $S=2.79$, $df=2$, $p=0.25$).

Experiment 3: how does the female sexual response to male song vary over the adult lifespan?

We found that the threshold amplitude of the male song stimulus eliciting female orientation remained relatively constant over the three test ages (Fig. 1c). Most tested females showed an orientation response when 1 day old (27 of 31 females) as well as at subsequent test ages, no female responded to the song stimulus broadcast at 57 dB peSPL, and all females that responded did so by the 75-dB amplitude level. Thus, we assumed that the few females that never oriented were non-receptive, as opposed to having a response threshold above the highest amplitude level presented (87 dB), and we eliminated these individuals from our analyses.

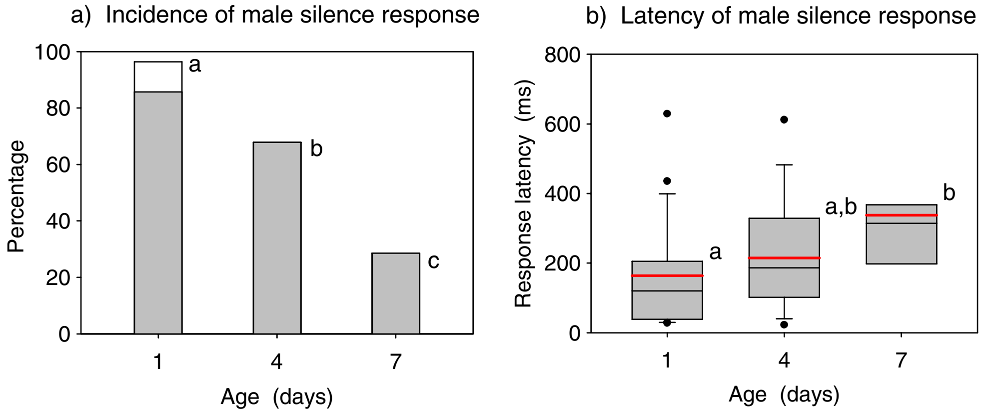

Fig. 2 Male silence response at three ages to a standard (85 dB SPL) bat echolocation signal. **a** Percentage of tested males exhibiting a silence response vs. age; *unshaded portion of bar* indicates males whose responses were shorter than 0.5 s. *Bars followed by the same letter* indicate values that are not significantly different [test of two binomial proportions (two-tailed), $\alpha=0.05$]. The letter '*a*' for the responses of 1-day-old males refers to all responses regardless of their length. **b** Latency of silence response vs. age. Box plots indicate mean (*solid red line*), median (*solid black line*), 25–75% range (*box*), 10–90% range (*whiskers*), and outliers. *Boxes followed by the same letter* indicate values that are not significantly different (Kruskal–Wallis test, analyzed by Dunn's method for multiple comparisons, $\alpha=0.05$)

The distributions of threshold amplitude levels of the male song stimulus that elicited an orientation response in female *A. grisella* did not differ significantly among the three age classes. Owing to small numbers of observations in many of the cells in the 3×3 contingency table (three threshold amplitudes×three female ages), we pooled our observations of 69- and 75-dB threshold amplitudes or of 63- and 69-dB threshold amplitudes in order to perform the requisite statistics. We observed no difference between 1- and 3-day-old females categorized by thresholds of 63 dB vs. 69 or 75 dB [McNemar test (two-tailed), $\chi^2=0.8$, $df=1$, $p=0.37$] or categorized by thresholds of 63 or 69 dB vs. 75 dB ($\chi^2=1.13$, $df=1$, $p=0.29$). Similarly, we observed no difference between 3- and 5-day-old females categorized by thresholds of 63 dB vs. 69 or 75 dB ($\chi^2=0.5$, $df=1$, $p=0.48$) or categorized by thresholds of 63 or 69 dB vs. 75 dB ($\chi^2=0$, $df=1$, $p=1.0$). Finally, no difference was observed between 1- and 5-day-old females for the same comparisons (63 dB vs. 69 or 75 dB—$\chi^2=0$, $df=1$, $p=1.0$; 63 or 69 dB vs. 75 dB—$\chi^2=1.13$, $df=1$, $p=0.29$).

Experiment 4: how does the male competitive response to male song vary over the adult lifespan?

As in the female sexual response tested in experiment 3, we found that the threshold amplitude of the male song stimulus eliciting a male competitive response remained relatively constant over the three test ages (Fig. 1d). Nearly all tested males (11 of 14, 14 of 14, and 16 of 17 of 1-, 4-, and 7-day-old males, respectively) exhibited a competitive response during the ascending series of the male song stimulus presented at each of the three ages. In each of these three age classes, only one male individual failed to respond. Distributions of threshold amplitude levels did not differ significantly between the three age classes (1 vs. 4 days old—*G* test, $\chi^2=0.747$, $df=2$, $p=0.69$; 4 vs. 7 days old— $\chi^2=0.36$, $df=2$, $p=0.84$; 1 vs. 7 days old—$\chi^2=0.17$, $df=2$, $p=0.92$).

Discussion

Results from our four experiments show that both female and male *A. grisella* become less sensitive to predator signals as they age, while sensitivity to sexual signals remains relatively constant. That is, the likelihood that anti-predator behavior is expressed—which may reduce mating opportunities for both sexes—declines, but basic mate seeking and competition for mates continue at standard levels. We observed no evidence of gradual senescence in the responses of females and males to male song, and we therefore reject the alternative hypothesis that physiological aging is directly responsible for the reduced sensitivity to predator signals in older individuals. Our results are consistent with the hypothesis that individuals engage in risk trading, the acceptance of increased exposure to predation as one ages chronologically in order to avoid the loss of terminal mating opportunities.

In rejecting the possibility that the decreasing sensitivity to predator signals reflects senescence, we considered that the same mechanical structure (tympanum) and peripheral (tympanic) neurons (Rodriguez et al. 2005) perceive both bat echolocation emissions and male song. Moreover, we note that both the synthetic bat echolocations tested here and *A. grisella* male song have overlapping frequency ranges and therefore would be processed in similar fashion at the peripheral level. Whereas these two categories of acoustic information are probably discriminated at the central level based on different temporal features and then processed along separate auditory streams (Greenfield and Hohendorf 2009; cf. Schul and Sheridan 2006; Höbel and Schul 2007), we do not *a priori* have any reason to expect a marked difference in senescence schedules between these streams in the central nervous system. We may also consider the possibility that the motor activities evoked by perceiving predator signals differ from those evoked by perceiving male song, which could lead to different senescence schedules in anti-predator and sexual behavior (Bernal et al. 2009). However, the anti-predator behaviors shown by females and males involve rather different motor tasks, arrestment of movement and cessation of song (wing fanning), respectively, yet both show the same elevation of response threshold with age. Similarly, the sexual behaviors shown by females and males, running toward male song and increased pulse-pair rate in song (wing-fanning rhythm), respectively, are quite dissimilar but retain the same response thresholds across adult age. Thus, an elevation of response threshold with age is related to behavioral context, anti-predator vs. mating activity, as opposed to gender or a specific motor task.

Does the purported risk trading in *A. grisella* afford maximum fitness, as would be predicted by general life history theory? A precise analysis would require observations of natural populations in the field, particularly interactions between *A. grisella* and predators such as insectivorous bats. However, we can offer some qualitative inferences based on our current understanding, which includes an ongoing flight-room study indicating that gleaning bat species (*Rhinolophus ferrumequineum*) perceive, investigate, and will feed on singing male *A. grisella* and that *A. grisella* females and males do exhibit arrestment and silence responses to bats flying and echolocating nearby (S. Alem, B. Siemers, K. Keselj, unpublished data). This knowledge suggests that a male who continues singing while perceiving bat echolocations may incur the attention of potential predators, but at the same time he may attract more females than neighboring males, who invariably exhibit an extended silence response, attract. Here, life history theory predicts that older males, who are less likely to exhibit this response, would benefit by accepting increased exposure to predation, as their expected opportunities for mating in the future are few.

From the female perspective, an individual who continues orienting toward a singing male when perceiving bat echolocations may also incur the risk of predation, while availing herself of mating with a particular male. Studies of mating behavior in *A. grisella* show that females engage in extensive sampling of local males (Alem and Greenfield 2010) and that they make precise evaluations of various song characters, singly (Jang and Greenfield 1996; Limousin and Greenfield 2009) and overall (Jang and Greenfield 1998, 2000). Moreover, these signal characters are generally heritable traits (Collins et al. 1999; Brandt and Greenfield 2004). Thus, a female who continues her mate sampling and evaluation in the face of predation may pair with a 'superior' singer and produce male offspring of superior attractiveness than a female who interrupts her activities and eventually mates with the nearest male, who might be of average 'quality'. However, we recall that female *A. grisella* become receptive shortly after the adult molt and normally mate but once, implying a small likelihood that 5-day-old (unmated) females, who are searching for males, exist in the field. Consequently, we ask whether females in natural populations would ever actually engage in risk trading toward the end of adult lifespan? Perhaps this event would be seen when population sizes are low, as may occur after resource patches (honeybee colonies and their organic detritus) are newly colonized by *A. grisella* or when these patches decline in resource value and few moths remain.

Studies of behavioral adjustment in accordance with expectations of current versus future reproduction are usually conducted using organisms, vertebrate or invertebrate, with lifespans considerably longer than that of *A. grisella* (but see Javoiš and Tammaru 2004). Finding that such adjustments occur during the course of an adult lifespan shorter than 10 days indicates that life history principles may operate regardless of the organism's longevity. In *A. grisella*, we did not observe that older females, and males, relaxed their discrimination of males by lowering their response threshold (Fig. 1c,d), but this threshold (approx. 70 dB SPL) may already be set at a physiological limit. However, both females and males showed a marked change in anti-predator behavior, with risk exposure increasing significantly over the 5–7 days following the adult molt. That *A. grisella* do not show major senescence over this interval (Jang et al. 1997; Brandt et al. 2005; cf. Bonduriansky and Brassil 2002, who report marked senescence over a short lifespan in natural populations of a fly species) suggests that the behavioral changes are controlled by an endogenous clock that runs largely on chronological age. Perhaps such temporal control reflects the overriding importance of absolute, chronological age in predicting expected future lifespan, and reproductive opportunities, in a short-lived insect where the probability of mortality follows a given schedule that is more or less independent of decline in various behavioral and physiological functions. It is our hope that the findings reported here inspire future studies of such internal chronological controls and how they may operate in the context of life history and the allocation of reproductive effort.

Acknowledgments We thank Guy Bourdais, Bruno Brizard, and Fabrice Vannier (I.R.B.I., Tours, France) for technical assistance in the laboratory, Bob Danka and Robin Cargell (U.S. Department of Agriculture, Baton Rouge, LA) for helping us to collect our Louisiana population of *A. grisella*, Bethany Harris and Yihong Zhou (University of Kansas) for helping to breed and rear the population, and the Agence Nationale de la Recherche (contrat ANR-07-BLAN-0113-01), the Centre National de la Recherche Scientifique (CNRS), the Université François Rabelais de Tours, and the U.S. National Science Foundation (IOB-0516634) for their financial support. We also thank Marlene Goubault, Séverine Ligout, Nathan Morehouse, Toomas Tammaru, and an anonymous referee for valuable criticisms of an earlier version of this manuscript.

References

Acharya L, McNeil JN (1998) Predation risk and mating behavior: the responses of moths to bat-like ultrasound. Behav Ecol 9:552–558

Alem S, Greenfield MD (2010) Economics of mate choice at leks: do female waxmoths pay costs for indirect genetic benefits? Behav Ecol 21:615–625

Arlettaz R, Jones G, Racey PA (2001) Effect of acoustic clutter on prey detection by bats. Nature 414:742–745

Bercovitch FB, Loomis CP, Rieches RG (2009) Age-specific changes in reproductive effort and terminal investment in female Nile lechwe. J Mammal 90:40–46

Bernal XE, Rand AS, Ryan MJ (2009) Task differences confound sex differences in receiver permissiveness in tungara frogs. Proc R Soc Lond B 273:1323–1329

Bonduriansky R, Brassil CE (2002) Senescence: rapid and costly ageing in wild male flies. Nature 420:377

Brandt LSE, Greenfield MD (2004) Condition-dependent traits and the capture of genetic variance in male advertisement song. J Evol Biol 17:821–828

Brandt LSE, Ludvar BC, Greenfield MD (2005) Co-occurrence of acceptance thresholds and preference functions in female choice: mate discrimination in the lesser wax moth. Ethology 111:609–625

Brinklov S, Kalko EKV, Surlykke A (2009) Intense echolocation calls from two 'whispering' bats, *Artibeus jamaicensis* and *Macrophyllum macrophyllum* (Phyllostomidae). J Exp Biol 212:11–20

Candolin U (1998) Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. Proc R Soc Lond B 265:1171–1175

Clutton-Brock TH (1984) Reproductive effort and terminal investment in iteroparous animals. Am Nat 123:212–229

Collins RD, Jang Y, Reinhold K, Greenfield MD (1999) Quantitative genetics of ultrasonic advertisement signaling in the lesser waxmoth *Achroia grisella* (Lepidoptera: Pyralidae). Heredity 83:644–651

Descamps S, Boutin S, Berteaux D, Gaillard JM (2007) Female red squirrels fit Williams' hypothesis of increasing reproductive effort with increasing age. J Anim Ecol 76:1192–1201

Ericsson G, Wallin K, Ball JP, Broberg M (2001) Age-related reproductive effort and senescence in free-ranging moose, *Alces alces*. Ecology 82:1613–1620

Fletcher JP, Hughes JP, Harvey IF (1994) Life expectancy and egg load affect oviposition: decisions of a solitary parasitoid. Proc R Soc Lond B 258:163–167

Fuller R, Berglund A (1996) Behavioral responses of a sex-role reversed pipefish to a gradient of perceived predation risk. Behav Ecol 7:69–75

Greenfield MD, Baker M (2003) Bat avoidance in non-aerial insects: the silence response of signaling males in an acoustic moth. Ethology 109:427–442

Greenfield MD, Coffelt JA (1983) Reproductive behaviour of the lesser wax moth, *Achroia grisella* (Pyralidae: Galleriinae): signalling, pair formation, male interactions, and mate guarding. Behaviour 84:287–315

Greenfield MD, Hohendorf H (2009) Independence of sexual and anti-predator perceptual functions in an acoustic moth: implications for the receiver bias mechanism in signal evolution. Ethology 115:1137–1149

Greenfield MD, Weber T (2000) Evolution of ultrasonic signalling in wax moths: discrimination of ultrasonic mating calls from bat echolocation signals and the exploitation of an anti-predator receiver bias by sexual advertisement. Ethol Ecol Evol 12:259–279

Greig EI, Greenfield MD (2004) Sexual selection and predator avoidance in an acoustic moth: discriminating females take fewer risks. Behaviour 141:799–815

Hedrick AV, Dill LM (1993) Mate choice by female crickets is influenced by predation risk. Anim Behav 46:193–196

Höbel G, Schul J (2007) Listening for males and bats: spectral processing in the hearing organ of *Neoconocephalus bivocatus* (Orthoptera: Tettigoniidae). J Comp Physiol A 193:917–925

Holm S (1979) A simple sequentially-rejective multiple test procedure. Scand J Stat 6:65–70

Isaac JL, Johnson CN (2005) Terminal reproductive effort in a marsupial. Biol Lett 1:271–275

Jang Y, Greenfield MD (1996) Ultrasonic communication and sexual selection in wax moths: female choice based on energy and asynchrony of male signals. Anim Behav 51:1095–1106

Jang Y, Greenfield MD (1998) Absolute versus relative measurements of sexual selection: assessing the contributions of ultrasonic signal characters to mate attraction in lesser wax moths, *Achroia grisella* (Lepidoptera: Pyralidae). Evolution 52:1383–1393

Jang Y, Greenfield MD (2000) Quantitative genetics of female choice in an ultrasonic pyralid moth, *Achroia grisella*: variation and evolvability of preference along multiple dimensions of the male advertisement signal. Heredity 84:73–80

Jang Y, Collins RD, Greenfield MD (1997) Variation and repeatability of ultrasonic sexual advertisement signals in *Achroia grisella* (Lepidoptera: Pyralidae). J Insect Behav 10:87–98

Javoiš J, Tammaru T (2004) Reproductive decisions are sensitive to cues of life expectancy: the case of a moth. Anim Behav 68:249–255

Jia FY, Greenfield MD, Collins RD (2001) Ultrasonic signal competition between male wax moths. J Insect Behav 14:19–33

Jones G, Barabas A, Elliot W, Parsons S (2002) Female greater wax moths reduce sexual display behavior in relation to the potential risk of predation by echolocating bats. Behav Ecol 13:375–380

Koga T, Backwell P, Jennions M, Christy J (1998) Elevated predation risk changes mating behaviour and courtship in a fiddler crab. Proc R Soc Lond B 65:1385–1390

Limousin D, Greenfield MD (2009) Evaluation of amplitude in male song: female waxmoths respond to fortissimo notes. J Exp Biol 212:4091–4100

Magnhagen C (1990) Reproduction under predation risk in the sand goby, *Pomatoschistus minutus*, and the black goby, *Gobius niger*: the effect of age and longevity. Behav Ecol Sociobiol 26:331–335

Neuweiler G (2000) The biology of bats. Oxford University Press, Oxford

Real L (1990) Search theory and mate choice. 1. Models for single-sex discrimination. Am Nat 136:376–404

Rodriguez RL, Greenfield MD (2004) Behavioral context regulates dual function of hearing in ultrasonic moths: bat avoidance and pair formation. Physiol Entomol 29:159–168

Rodriguez RL, Schul J, Cocroft RB, Greenfield MD (2005) The contribution of tympanic transmission to fine temporal signal evaluation in an ultrasonic moth. J Exp Biol 208:4159–4165

Roff DA (1992) The evolution of life histories, theory and analysis. Chapman & Hall, New York

Schul J, Sheridan RA (2006) Auditory stream segregation in an insect. Neuroscience 138:1–4

Sirot E, Ploye H, Bernstein C (1997) State dependent superparasitism in a solitary parasitoid: egg load and survival. Behav Ecol 8:226–232

Spangler HG, Greenfield MD, Takessian A (1984) Ultrasonic mate calling in the lesser wax moth. Physiol Entomol 9:87–95

Stearns SC (1992) The evolution of life-histories. Oxford University Press, Oxford

Waters DA (2003) Bats and moths: what is there left to learn? Physiol Entomol 28:237–250

Waters DA, Jones G (1995) Echolocation call structure and intensity in five species of insectivorous bats. J Exp Biol 198:475–498

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, Upper Saddle River

Zuk M, Kolluru G (1998) Exploitation of sexual signals by predators and parasitoids. Q Rev Biol 73:415–438