

Economics of mate choice at leks: do female waxmoths pay costs for indirect genetic benefits?

Sylvain Alem and Michael D. Greenfield

Institut de recherche sur la biologie de l'insecte, Centre National de la Recherche Scientifique Unité Mixte de Recherche 6035, Université François Rabelais de Tours, Parc de Grandmont, 37200 Tours, France

Sexual selection theory predicts low costs of choice when females choose among males for genetic (indirect) benefits, as occurs at leks. However, few empirical studies have investigated the actual costs incurred during the process of pair formation, and we generally do not know whether and to what extent females incur energetic expenditure, exposure to predation, or simple allocation of time by virtue of their choosiness. Do females that choose mates at larger aggregations of males necessarily pay these costs? Moreover, what costs are they willing to pay to obtain potential benefits? We addressed these questions in an acoustic pyralid moth, *Achroia grisella*, in which males aggregate and attract females with an advertisement song. Female choice is based on acoustic characters of displaying males, and only genetic benefits appear to be available. We measured the movement and time that females spent in mate sampling when presented with varying numbers of males in a laboratory arena. We found that female choice for specific males was retained as male number increased, although their sampling effort increased: female trajectories lengthened and reversed direction more often. The repeatability of female choice at larger leks and the basic precision of female phonotaxis indicated that the lengthened trajectories reflected sampling and choosiness as opposed to confusion. We propose that the cost of such choosiness in natural populations may be an increased exposure to predation and that females pay this cost because of the opportunity to mate with a specific male with certain song characteristics. *Key words*: acoustic communication, mating signals, orientation, pair formation, sexual selection. [*Behav Ecol* 21:615–625 (2010)]

The costs and benefits that accrue to individuals engaged in courtship and mating represent an integral part of the sexual selection process (Andersson 1994). Although these economic factors can be expected to operate in the activities of both sexes, they were first considered and have been more thoroughly studied in the male. Following this scheme, modeling of the indirect (genetic) benefits mechanism of mate choice has generally considered that as a male's signaling increases in extravagance, his elevated costs incurred in energy expenditure or exposure to the risk of predation are offset by increased attractiveness to females (Kotiaho 2001; Stuart-Fox et al. 2003; Danchin and Cézilly 2005; e.g., Reinhold et al. 1998). Analyses of female activities, however, focused initially on the various direct and indirect benefits that might be received by virtue of mating with a given male or with a given number of males, while ignoring potential costs or assuming that they are negligible. More recently, though, various modelers have proposed that females might sustain certain costs of choice when they receive direct benefits (Kirkpatrick 1985, 1996; Heywood 1989; Grafen 1990; Kirkpatrick and Ryan 1991; Kirkpatrick and Barton 1997; Cameron et al. 2003), and even in the case of indirect benefits provided that the costs are small (Iwasa et al. 1991; Pomiankowski et al. 1991; Houle and Kondrashov 2002; Kokko et al. 2006). Similarly, a parallel series of empirical studies have begun to address the costs that females actually encounter in the process of pair formation (Parker 1978; Jennions and Petrie 1997; for studies

that have measured such costs, see Wickman and Jansson 1997; Byers et al. 2005; Booksmythe et al. 2008). Nonetheless, basic questions remain because we still have relatively little information on which aspects of the entire pair-forming process are costly for a female and whether choosy females might receive certain added benefits that offset those costs. Empirical studies are particularly critical in species with no evidence for direct benefits, as theory predicts that mate choice under these circumstances should be relatively cost free, but some observations suggest otherwise.

It has generally been recognized that aggregations of lekking males are ideal settings for studying female choice for indirect benefits (Höglund and Alatalo 1995). These same aggregations may also serve as most appropriate settings for investigating the costs associated with pair formation via female choice. We consider that the activity of choosing to mate with a particular male displaying at a lek may be partitioned into 2 processes, mate preference and "choosiness" (see Jennions and Petrie 1997; Kokko et al. 2006). We retain the distinction between these 2 processes because each may function independently within the context of pair formation. For example, 2 females may have the same "preference functions" (see Ritchie 1996) along the male phenotypic gradient, but one may have a high level of choosiness wherein she spends considerable time and effort in evaluating available males before pairing, whereas another being less choosy might pair more quickly, and possibly with a male displaying inferior courtship. In other words, the second makes a more cursory examination and in effect either adjusts her acceptance threshold to a lower value or has a higher probability of mating with an inferior male due to limited sampling (see Janetos 1980).

We assume that the costs of female choice are largely subsumed within choosiness rather than preference unless there are costs associated with mating with specific categories of

Address correspondence to S. Alem. E-mail: sylvain.alem@etu.univ-tours.fr.

Received 14 September 2009; revised 9 February 2010; accepted 12 February 2010.

males (e.g., Head et al. 2005). In the context of leks, a female which visits or otherwise perceives a fixed proportion of displaying males and effects a given evaluation of each male will pay a greater cost when encountering leks of increasing male number (see Janetos 1980). Alternatively, she could avoid these elevated costs by visiting or perceiving a smaller proportion or reducing the evaluation of each male when encountering larger leks (Kotiaho and Puurtinen 2007). Thus, we might experimentally manipulate lek size, effectively changing the cost of choosiness, and then examine the effect on female sampling behavior and mate choice. Because costs associated with mate sampling are potential constraints on the optimal mate choice (Gibson and Bachmann 1992), we can observe whether females use “cost-reducing tactics,” as some theoretical models predict (see Höglund and Alatalo 1995) or endure additional costs at larger leks to mate with a specific male. And by examining female choice at these experimental leks, we can also observe whether varying costs of choosiness influence the intensity and directionality of sexual selection and whether the characteristics of males chosen at larger leks differ from those at smaller ones. That is, does the added cost of sampling at larger leks ultimately yield certain benefits?

We approached the above questions on the cost of choosiness through a series of experiments with an acoustic moth, *Achroia grisella* (lesser waxmoth; Lepidoptera: Pyralidae), in which singing males gather in small leks and attract receptive females (Spangler et al. 1984). Extensive laboratory studies of *A. grisella* have shown that females evaluate males primarily on the basis of song characters (Jang and Greenfield 1996, 1998) that such evaluation occurs independently of male–male competition (Cremer and Greenfield 1998) and may involve extensive movement among singing males and that males do not transfer direct material benefits at the time of mating (Greenfield and Coffelt 1983). However, females may receive indirect genetic benefits from mating with a specific male as several male song characters that influence attractiveness to females (Jia and Greenfield 1997; Collins et al. 1999; Jia et al. 2000), as well as overall song attractiveness (Brandt and Greenfield 2004), have been found to be heritable in various *A. grisella* populations. With this understanding, we proceeded to examine the mate choices that females made when presented with leks comprised of different numbers of males and of different individuals. Here, we analyzed the movement trajectories that females made in the course of their choice. We also noted the identity and song characters of the males that females chose among those present in these various leks.

We report that female *A. grisella* retain the same level of choosiness when visiting leks of greater male number and thereby spend more effort on mate evaluation in this situation. Moreover, females do not decrease this relative effort exhibited during visits to larger leks when they are exposed to synthetic signals of predators. Importantly, we note that our experiments measured the relative cost of choosiness that a female incurred in visiting larger leks rather than an absolute cost incurred in a specific situation, which would be more difficult to determine. We interpret our findings to indicate that females pay the added costs of mate choice for the opportunity to pair with a particular male. More generally, we note that the basic activity of choosing a mate has some economic price that is associated with sampling effort. We then discuss what this price might be in natural populations. Finally, we consider that by retaining a certain level of choosiness in mate choice and paying the added costs incurred in evaluating larger leks, females may obtain certain benefits by mating with males of higher “quality” than they would otherwise.

MATERIALS AND METHODS

Achroia grisella natural history; acoustic and reproductive behavior

Achroia grisella are symbionts of the western honeybee (*Apis mellifera*) and are found in most geographic regions of the world where honeybees are kept (Künike 1930). The larvae of *A. grisella* feed on brood, comb, and organic detritus within and surrounding honeybee colonies that have low worker populations and are therefore in a weakened condition. Adult *A. grisella* normally remain in the vicinity of the natal site provided that some resources remain, and mating activities take place in, on, or near the colony (Greenfield and Coffelt 1983).

Male *A. grisella* generate a high-frequency song by fanning their wings at 40–50 s⁻¹ while remaining stationary on the substrate (Spangler et al. 1984). This activity causes a pair of tymbals at the bases of the front wings to resonate, once on the wing upstroke and once on the downstroke. Each resonance yields a brief ($\approx 100 \mu\text{s}$) pulse of 100 kHz sound whose peak amplitude is ≈ 70 dB peSPL (peak equivalent sound pressure level; 0 dB = 20 μPa) at 20 cm. Because the activity of the left and right tymbals is not precisely synchronous, each wing upstroke and downstroke is normally represented by a pair of pulses wherein the onsets of the separate pulses are offset by a small (100–1000 μs) “asynchrony interval.” Thus, *A. grisella* song may be characterized by a continuous train of pulse pairs delivered at 80–100 s⁻¹ (at 25 °C). These acoustic displays attract receptive females up to 1-m distant, which normally run toward the males, move among them, and orient toward a given individual. Playback experiments using broadcasts of synthetic male song (Jang and Greenfield 1996; Limousin and Greenfield 2009) as well as live males (Jang and Greenfield 1998) showed that females prefer songs that include pulse pairs of greater peak amplitude, that have a faster repetition rate, that include longer asynchrony intervals, that have greater acoustic power (= product of pulse-pair rate \times mean peak amplitude; see Greig and Greenfield 2004), and certain overall combinations of these features. Males normally sing more or less uninterruptedly for 6–10 h on each night after their adult molt. Observations in the laboratory and field suggest that singing males may gather in small aggregations within which aggressive interactions may occur (Greenfield and Coffelt 1983; Cremer and Greenfield 1998). Despite these aggressions, when females visit male aggregations they freely choose among the individuals displaying there.

Additional playback experiments show that both male and female *A. grisella* respond to broadcasts of pulsed ultrasound that mimic the echolocations of insectivorous bats, including the echolocations of bat species that passively localize prey by listening to their sounds and then glean them from the substrate. *Achroia grisella* in flight drop to the ground when exposed to these synthetic echolocations (Rodriguez and Greenfield 2004), whereas singing males and females orienting toward males become silent and cease movement, respectively (Greenfield and Weber 2000; Greenfield and Baker 2003; Greig and Greenfield 2004; Greenfield and Hohendorf 2009).

The adult longevity of *A. grisella* is markedly short. On average, females and males kept at 25 °C survive only 7 and 10–14 days, respectively, and they neither feed nor drink (Greenfield and Coffelt 1983). Females usually mate only once and become unreceptive thereafter. Males, however, may mate once per day. There is no evidence that females obtain direct benefits such as spermatophore resources by virtue of mating with a given male. For example, female fecundity does not decline as a function of the number of times that the male has previously mated (cf. Bissoondath and Wiklund 1996).

Population studied

We studied an *A. grisella* laboratory population developed from several hundred larvae collected at infested honeybee colonies in Département Indre et Loire, France (47°19'N, 0°46'E) in October 2007. We reared the larvae on a standard diet comprised flours, honey, beeswax, glycerol, nutritional yeast, and water (see Jang and Greenfield 1996) and maintained them in an environmental chamber at 25 °C under a 12:12 light:dark photoperiod. Under these conditions, generation lengths averaged 45 days.

General procedure

To study the behavior of females arriving at leks of various sizes, we used a circular, screened arena (80-cm diameter) within which we placed different numbers of singing males in individual screen cages (2-cm diameter, 2-cm height) situated around the arena center (Figure 1). Females were released individually at the arena center, and their movements among the males were recorded with an overhead video camera. These video recordings were later analyzed to determine parameters of the females' trajectories. We also recorded and analyzed the songs of the males placed in the cages to assess the potential influence of song variation within a lek on female choosiness.

Experiment 1: do female orientation and evaluation of males change as a function of lek size?

To test whether female choosiness is costly and whether females pay these costs, we followed the responses of 27 females to groups of 2 and 4 males, hereafter termed 2-male and 4-male leks. Tested females were unmated and were between 1 and 4 days past their adult molt. Each was tested in a total of 6 trials, 3 trials with a 2-male lek and 3 trials with a 4-male lek. We conducted all 6 trials of a given female during the same day, but we allowed a minimum of 30 min to elapse between consecutive trials in order to reduce the possibility of habituation. The 6 trials were completed during the initial 6 h of the night, the interval of natural activity in *A. grisella*. We ordered a female's trials with the 2-male and 4-male leks in a random sequence.

On a given day, we presented leks comprised by the same groups of 2 and 4 males to the several females tested on that day. For consistency, we only sampled unmated males aged less than 7 days and that sang continuously to comprise these leks. We monitored the sounds of the presented males regularly

with a bat detector (Ultrasound Detector D230; Petterson Elektronik AB; Uppsala, Sweden) and thus verified that all individuals were singing before each trial. Previous studies (Jang and Greenfield 1996) have shown that the screen cages do not modify the acoustic characters of the male song broadcast outside. At the beginning of a test day, we arbitrarily designated 2 of the 4 presented males to represent the 2-male lek. We retained this designation in all trials on that day, and we removed the other 2 males from the test arena during the presentations of this 2-male lek. A given male was used on only one test day. Thus, a total of 56 males were presented on the 14 days during which the 27 females were tested.

We conducted all trials in an acoustically insulated environmental chamber kept at 25 °C and illuminated by diffuse red light from a 25-W incandescent bulb. The screen cages holding the 4 males were placed along the 4 cardinal directions radiating from the arena center, each cage situated 24 cm from that point (Figure 1). When the 2-male lek was presented, the individuals were placed at opposite directions from the arena center, whereas empty screen cages were retained at the 2 other directions. We alternated the positions of the male cages in the arena between successive trials to reduce potential confounding influences of side biases in female orientation. When the 4-male lek was presented, we also randomly changed the relative positions of the several individuals after each trial.

The apparatus for releasing the female consisted of a 2-cm diameter covered depression in the arena floor. Females were introduced to this depression, where they generally remained on the bottom owing to a coating of Teflon on the sides and ceiling, and allowed 3 min to adjust to their introduction. We then carefully removed the cover, so as to avoid disturbing the males singing in the surrounding cages, and simultaneously raised the bottom of the depression until it was level with the arena floor. Females were allowed 5 min to leave the release point, move within the arena, and arrive within 2 cm of a male cage and remain in that zone for a minimum 5 s, which signified the end of the trial. We kept the females within an acoustically insulated box between successive trials to prevent their continuous exposure to males and male song.

Observations in experiment 1 indicating that females follow a longer and more convoluted trajectory when presented with 4-male leks than with 2-male leks (see RESULTS) might reflect 1) the payment of additional costs for evaluating males in a larger lek, but they could also arise from 2) perceptual "errors" due to confusion. To distinguish these 2 possibilities, we analyzed the repeatability of choices that females had made

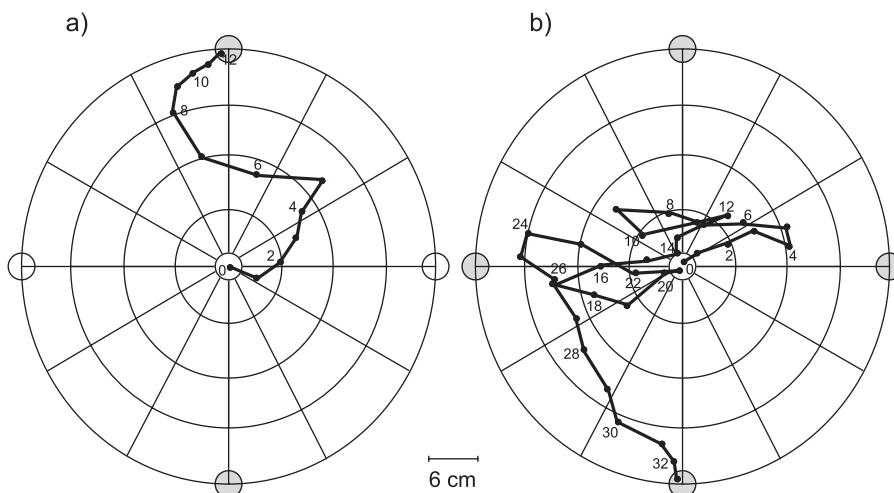


Figure 1
Examples of female orientation trajectories when presented with 2 singing males (a: trajectory length = 31.1 cm; duration of orientation = 12.0 s) and with 4 singing males (b: trajectory length = 102.2 cm; duration of orientation = 32.2 s). Numerals along trajectories indicate time (s) when the female passed the designated point. Shaded circles represent cages holding a male; open circles represent empty cages.

at the 4-male leks. If females cannot distinguish males at a larger lek because of confusion, the repeatability of a given female's choices should be low, and her overall pattern of choice should approach the random expectation. Thus, we tabulated the numbers of females which chose a different male individual in each of their 3 trials with the 4-male lek, the numbers which chose the same male twice, and the numbers which chose the same male in all 3 trials, and then compared this observed distribution with that expected were mate choices random. We applied this same procedure to analyze the repeatability of female choice at 2-male leks.

Experiment 2: do female choices of males change as a function of lek size?

To evaluate whether females benefit from visiting larger male aggregations, we examined the consistency of a female's choices at leks of different size. We tested the possibilities that females do not necessarily choose the same male when additional males are available owing to 1) greater difficulty in evaluating all males in a larger group or 2) because the larger group may include some males of superior quality. Here, findings indicating that females generally choose the same male in leks of different size would provide additional support for the alternative hypothesis that females pay an elevated cost of choice at a larger lek. Moreover, findings indicating that the occasions when females do choose a different male in the 4-male lek generally involve choice of a superior singer (see Acoustic recording and analysis section below) may bolster that alternative hypothesis and specify the nature of the benefits forthcoming from their choosiness.

To test these predictions, we repeated the above experiment except that the females were tested only twice, once with a 2-male lek and once with a 4-male lek, and the second trial invariably included the male chosen in the first trial. To avoid the effect of sequence, one-half of the females were tested first with the 2-male lek, whereas the other half were tested first with the 4-male lek.

For the females tested first with a 2-male lek, the 2 males were randomly drawn from the 4 males used on that day and were placed on opposite sides of the arena to comprise that group. We retained these 2 males on opposite sides of the arena in the female's second trial, where a 4-male lek was presented. However, the specific directions along which the males were placed were randomly assigned in each trial. The random expectation that the female chooses the same male in the second trial is 0.25.

For the females tested first with a 4-male lek, we used the male chosen from that group and the male opposite him for the second trial, in which a 2-male lek was presented. As above, these 2 males were kept on opposite sides of the arena but the specific directions along which they were placed in the arena were randomly assigned in each trial. Here, the random expectation that the female chooses the same male in the second trial is 0.50.

Each of 63 different females was tested on the day of adult eclosion, whereas the males were between 0 and 6 days old on the day of testing. We presented a total of 56 males on 14 test days and used a given male on only one test day.

Experiment 3: what is the efficiency of acoustic orientation in females?

If females effect longer trajectories at 4-male leks (see RESULTS, experiment 1) and do so to improve their evaluation, they should exhibit not only repeatability in their choice but also a fundamental precision in their acoustic orientation. That is, an ability to move toward an acoustic stimulus in a di-

rect line would be predicted. We therefore conducted an additional experiment that measured the basic phonotactic precision of a female *A. grisella* during orientation to a single singing male. We tested 15 females, each aged 1 day, in 4 trials within the arena wherein we placed only one male in a screen cage 24-cm distant from the release point. As in experiments 1 and 2, we allowed a minimum 30 min to elapse between a female's successive trials. We used a different male, aged 1 day, in each trial. The female's trajectory was video recorded until 20 s had elapsed after her release. In the DISCUSSION, we compare the observed phonotactic precision of female *A. grisella* with various acoustic insects where such data exist.

Experiment 4: how do females evaluate male leks when exposed to predator signals?

If females do spend more time and energy in evaluating larger leks (see RESULTS, experiment 1), does this effort represent an actual cost in natural populations? Because one potential cost of extended evaluation is greater exposure to predation, we conducted a final experiment in which females were exposed to synthetic bat echolocations while visiting 2-male and 4-male leks. Thus, we observed whether females continued their evaluation of males at larger leks under the risk of potential predation or whether they ceased movement, their typical response when orienting toward a loudspeaker broadcasting the song of a single male (Greenfield and Weber 2000).

Our experiment on exposure to predation risk used the same procedure as in experiment 1 except that synthetic bat echolocation signals were broadcasted from an overhead loudspeaker. The signals were 2-ms pulses whose carrier frequency was modulated (FM) to sweep downward from 100 to 25 kHz over the 2-ms length. We repeated these FM pulses at a rhythm of 15 s^{-1} and adjusted their amplitude, as measured at the location of the female, to 95 dB peak equivalent SPL (0 dB = 20 μ Pa; see Greenfield and Hohendorf 2009 for details on pulse generation and justification for representing bat echolocation signals with pulses bearing these acoustic parameters). We initiated the broadcast of synthetic echolocation signals after the test female had moved several centimeters from the central release point and continued it for 5 s. Because males in the small screen cages within the arena might become silent when exposed to synthetic echolocation signals, we placed a roof of acoustic foam over each male cage. Thus, males were protected and continued to sing, whereas females were exposed to both male song and the synthetic echolocation signals.

We tested 22 females on the day of their adult molt. Each was tested in 4 trials, 2 with a 4-male lek and then 2 with a 2-male lek. The same protocol used in experiment 2 was followed to comprise the 2-male leks. For the 2 trials of a 4-male lek and for the 2 trials of a 2-male lek, one trial included the broadcast of synthetic echolocation signals and one did not. The order of these 2 trials was rerandomized for each female. The same 4 male individuals were used to comprise the leks for each of the 22 females tested.

Video recording and trajectory analysis

We recorded each trial in the first 3 experiments with a Sony HD-7RSE video recorder operated in the "nightshot mode." The recorder took 25 images per second and was placed at a height such that each image covered the entire arena. The floor of the arena was covered with white paper marked with grid lines to render the female's posture and movement more visible.

Following recording in experiments 1 and 2, we analyzed the female's trajectory either manually or with video tracking

software (Viewer; Biobserve; Bonn, Germany) that automatically provided the x and y coordinates of her position at regular intervals. Software (MB Ruler; MB Software Solutions [Markus Baker]; Germany) for determining distances and coordinates on a computer screen aided our manual analysis. The data provided were then used to determine the approximate length of the female's trajectory between release and arrival within the 2-cm zone around a male cage, the duration of this trajectory, and the characteristics of her "turn angles" during the trajectory. We measured turn angles by taking the female's azimuth heading (α_t) at the end of each 1-s interval, defined by the straight line connecting her coordinates at times t and $t - 1$ s, and then computing the difference between 2 consecutive azimuth headings ($\alpha_{t+1} - \alpha_t$) (Figure 2a). Thus, a trajectory that lasted t s would be characterized by $t - 1$ turn angles. We determined the mean turn angle of the trajectory in each trial, as well as the number of turn angles that exceeded a given threshold value, either 45° or 90° . Because large turn angles during a female's trajectory could represent her continuing evaluation of the several males present in the arena, we considered the turn angle statistics as indices of her choosiness. We also considered that the final turn angle that exceeded a threshold value marked the point of a female's final choice of a male, and we therefore noted the duration and length of her trajectory between release and that point, as determined with either the 45° or 90° criterion. These angular criteria were used because *A. grisella* females normally do not orient directly toward a male until the final centimeters.

In experiment 3, we used a somewhat different procedure to analyze the precision of female orientation toward a singing male. At 3 points during a female's trajectory toward a male, when 18-, 12-, and 6-cm distant, we measured the azimuth of her heading (designated angle β , measured at time = t) relative to the direction toward the male and her subsequent change of direction (designated correction angle γ , measured at time = $t + 240$ ms, 6 images later; this interval was experimentally determined to be the most informative one for our analysis) relative to that initial heading (Figure 2b). This correction angle thus represented a female's ability to lateralize the singing male rapidly were she off-course. We then graphed the correction angle as a function of the initial heading to examine whether the magnitude and sign of a female's turn toward the male were commensurate with her initial deviation from him.

Acoustic recording and analysis

In experiments 1 and 2, we recorded the song of each male twice, at the beginning and end of the day's testing period. Previous studies showed that the acoustic parameters of male *A. grisella* song are quite repeatable within a day (Jang et al. 1997; also see Brandt et al. 2005), making more regular recording unnecessary. Recordings of 10-s duration were made with a condenser microphone (model CM16/CMPA; Avisoft Bioacoustics; Berlin, Germany; frequency response: ± 3 dB, 20–150 kHz) held at a standard distance (24 cm) from the male, digitized with an analogue/digital converter at 16 bits and 192 000 samples s^{-1} (TASCAM HD-P2 recorder; TEAC Corp.; Tokyo, Japan), and saved as sound files. We later determined the pulse-pair rate, mean peak amplitude (measured on a relative, linear scale), and acoustic power (= pulse-pair rate \times mean peak amplitude) of each recording (see Greig and Greenfield 2004). We then averaged a male's 2 values for each parameter to estimate his mean pulse-pair rate, peak amplitude, and acoustic power. We used these mean values to evaluate the song characteristics of the males that females chose in leks of different size (experiment 1). The values were also used to evaluate whether females that chose different individuals in the 4-male and 2-male leks (experiment 2) did so because the former included a males with superior acoustic characteristics.

In the context of choosiness and its measurement by trajectory length, duration, and turn angles, we anticipated that these indices might be influenced by the specific acoustic characteristics of the males comprising the leks as well as by male number. For example, choice of a specific male might be more difficult—and require a lengthier trajectory—in a lek wherein each individual sang with similar acoustic characteristics than in a lek wherein 1 or 2 males were clearly superior. Thus, we measured an index of acoustic power diversity, calculated as the difference in acoustic power between the males in each 2-male lek and as the mean of the 6 pairwise differences in acoustic power in each 4-male lek. This latter calculation reflected our assumption that females make binary decisions when evaluating males. We used these measures of acoustic power diversity, as well as the variance of acoustic power in 4-male leks, to disentangle any confounding influences of specific lek composition on choosiness (experiment 1) as well as on consistency of choice (experiment 2).

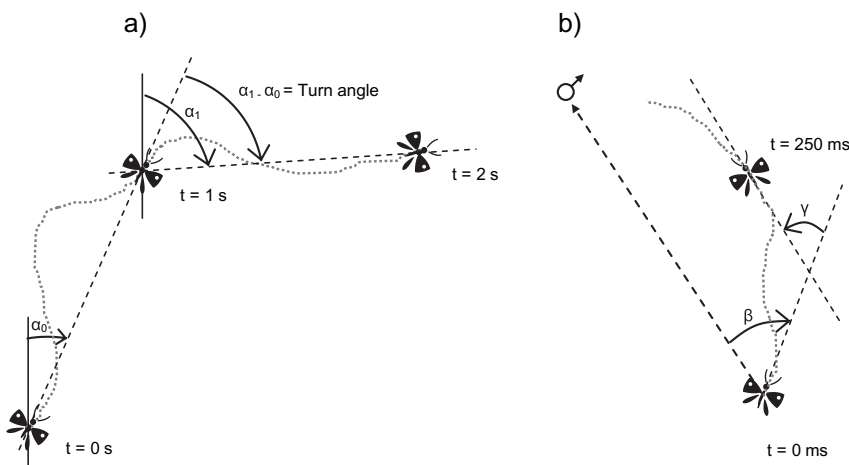


Figure 2

Method of calculating turn angles that describe female orientation at a lek (a; experiment 1) and toward a single singing male (b; experiment 3). Dotted lines represent the female trajectory; points along these lines represent female position at designated times; dashed lines represent female orientation. (a) Orientation at a lek (experiment 1), showing turn angle calculated for $t = 0$ s ($\alpha_{t+1} - \alpha_t$). (b) Orientation toward a single singing male (experiment 3), showing azimuth of female heading relative to male at time 0 ms (angle β) and correction angle in female's heading made over the subsequent 240-ms interval (angle γ). Not drawn to scale.

RESULTS

Experiment 1: do female orientation and evaluation of males change as a function of lek size?

Analyses of female orientation in the test arena showed that most individuals lengthened their trajectories in the presence of larger leks. Of 27 females tested, 22 oriented to a specific male in each of their 6 trials; the remaining 5 females were not used in our analyses. For these 22 females that oriented regularly, we found that total trajectory length and duration rose significantly as lek size was increased from 2 to 4 males (Figures 1 and 3a,b). The length and duration of the trajectory measured until the final choice of a male, whether estimated by a 45° or 90° threshold angle, also rose significantly at 4-male leks (Figure 3a,b). Females also made more turns that exceeded a 45° or 90° threshold angle at 4-male leks (Figure 3c), and the mean turn angles made during the course of their trajectories were greater when orienting at 4-male leks than at 2-male leks (Figure 3d).

We found, however, that neither the acoustic power diversity nor variance measured among the males presented in the test arena influenced any of the above trajectory indices (Spearman rank correlation; $P > 0.10$). The absence of a relationship between acoustic power diversity and the trajectory a female followed held for both 2- and 4-male leks.

In examining the acoustic characteristics of the individual males that females chose during the trials in the arena, we found rather complex results. We first considered the trials with 2-male leks, and for each of the 3 acoustic characteristics measured, we divided the 22 test females into 2 groups, those that chose the male ranked first in 2 or 3 of their trials and those that chose him in only 0 or 1 of their trials. For all 3 acoustic characters, we found that the number of females preferring the first-ranked male in 2 or 3 of their trials did not significantly exceed the number preferring him in only 0 or 1 trial (binomial tests, 1-tailed; $P > 0.50$). However, when we removed from analysis those females (10/22) which had evaluated 2 acoustically similar males (amplitude and acoustic power

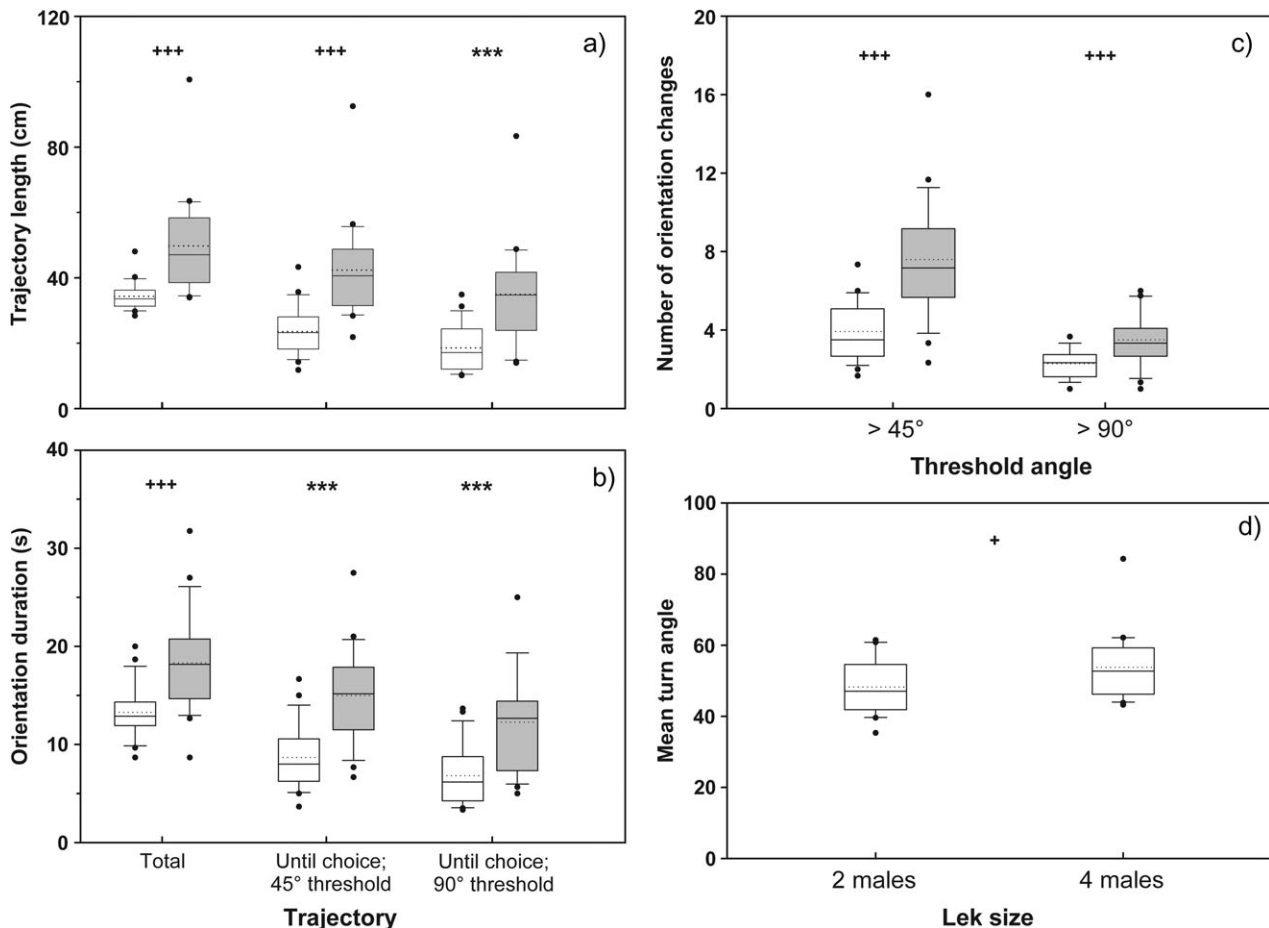


Figure 3

Analyses of orientation trajectories of the 22 females that oriented toward a male in each of 6 trials (experiment 1). Box plots indicate mean (dotted line), median (solid line), 25–75% range (box), 10–90% range (whiskers), and outliers. (a) Total length, length until the last 45° turn angle, and length until the last 90° turn angle of trajectories by females at 2-male (open boxes) and 4-male leks (shaded boxes). (b) Total duration, duration until the last 45° turn angle, and duration until the last 90° turn angle of trajectories by females at 2-male (open boxes) and 4-male leks (shaded boxes). (c) Number of turn angles exceeding 45° (open boxes) and exceeding 90° (shaded boxes) in trajectories by females at 2-male and at 4-male leks. (d) Mean turn angle of females at 2-male and at 4-male leks. In all cases, data represent the average value of a female's orientation in her 3 trials at a 2-male lek and her average value in her 3 trials at a 4-male lek. Paired *t*-test (2-tailed) as applied to within-female comparisons where data satisfied the requirements of normality and equality of variance; H_0 : average_{2-male lek} = average_{4-male lek}; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Wilcoxon signed-rank test (2-tailed) as applied to within-female comparisons where data did not satisfy the requirements of normality and equality of variance; H_0 : average_{2-male lek} = average_{4-male lek}; + $P < 0.05$; ++ $P < 0.01$; +++ $P < 0.001$.

differences between the 2 males were <10%), we found that the majority of the remaining females (9/12) chose the male singing with higher peak amplitude or with greater acoustic power in 2 or 3 of their trials ($P < 0.05$). Reconsidering those 10 females that had been removed from analysis, we observed that 7 of them chose the male singing with a faster pulse-pair rate in 2 or 3 of their trials ($P = 0.05$). We analyzed the trials with 4-male leks in a similar fashion, and for all 3 acoustic characters, we again found that the number of females preferring the first- or second-ranked male in 2 or 3 of their trials did not significantly exceed the number preferring such high-ranked males in only 0 or 1 trial ($P > 0.30$). But, if we consider the 13 females which had not chosen the male ranked either first or second for peak amplitude in 2 or 3 of their trials, we found that 10 of these females had chosen the male ranked first or second for pulse-pair rate in 2 or 3 of the trials ($P < 0.02$). That is, females generally chose males with superior acoustic characteristics, but individual females differed in which characteristic they evaluated more strongly.

We found that the pattern of successive choices of males by females visiting the 4-male leks differed significantly from the random expectation (Figure 4). Fewer females than expected chose a different male in each of their 3 trials, whereas more females than expected chose the same male in each trial. At 2-male leks, however, we found that the pattern of successive choices of males did not differ from the random expectation ($n = 22$, $\chi^2 = 1.53$, degrees of freedom [df] = 2, $P = 0.005$).

Experiment 2: do female choices of males change as a function of lek size?

We found that 68% of the 63 tested females chose the same male individual whether he was presented as part of a 2-male lek or part of a 4-male lek. Among the females for which a 4-male-lek was tested first, 76% of them chose the same male from the 2-male lek (expected proportion = 0.50; binomial test, 2-tailed; $P = 0.002$), whereas among the females for which a 2-male lek was tested first, 60% of them still chose the same male from the 4-male lek (expected proportion = 0.25; binomial test, 2-tailed; $P < 0.001$).

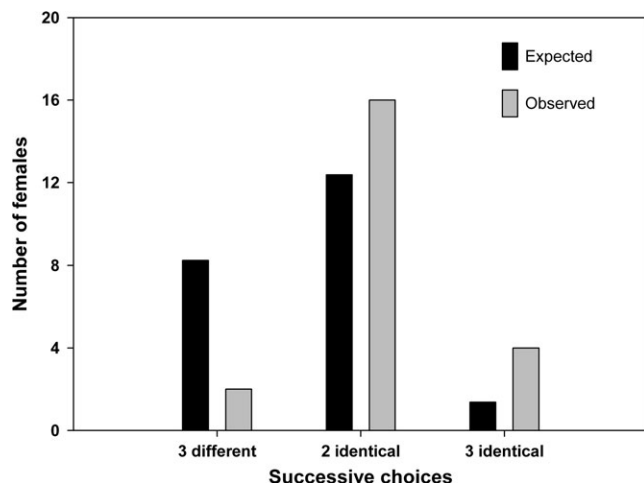


Figure 4 Repeatability of female choice at 4-male leks (experiment 1). Gray vertical bars represent the numbers of females ($n = 22$) that chose a different male in each of their 3 trials, chose the same male twice, and chose the same male in all 3 trials. Black bars represent the random (multinomial) expectation, assuming independence of successive choices. (H_0 : choices in the 3 trials differ from the random expectation; $\chi^2 = 10.8$, df = 2, $P = 0.005$).

We then considered the females for which a 2-male lek was tested first (30/63) and focused only on those individuals (12/30) that chose a different male in their second test, wherein a 4-male lek was presented. Contrary to one prediction, these females did not necessarily select superior singers when choosing a different male in their second test: The male chosen from the 4-male lek did not differ significantly from the first male chosen in acoustic power, amplitude, or pulse rate of his song (Figure 5).

As in experiment 1, acoustic power diversity within the leks did not appear to influence the probability that a female chose a different male in her second test: Females that were initially presented with 2 males with comparable song features—between whom a choice might have been somewhat random—were not more likely to choose a different male from the 4-male lek than females initially presented with 2 rather dissimilar males were (Figure 6).

Experiment 3: what is the efficiency of acoustic orientation in females?

In our test of the precision of orientation toward a single male, we observed that a female's deviation from a direct heading (angle β) was approximately $\pm 27^\circ$ (Figure 7a; average of all values at 18-, 12-, and 6-cm distances from the male). Females responded to these deviations by rapidly changing their heading (correction angle, γ) toward the male. The magnitude of these corrections was commensurate with the initial deviation at all 3 distances where we took measurements (Figure 7b,c,d). Thus, females appear to have the ability to lateralize a singing male so that he is directly ahead, but overcompensations in their correction angles (i.e., $|\gamma| > |\beta|$) often lead them to zigzag during their approach. We did not observe a decrease in the deviation angle (β) as a female approached the male (Kruskal-Wallis test; $P = 0.64$).

Experiment 4: how do females evaluate male leks when exposed to predator signals?

We found that females did not exhibit a greater tendency to cease movement during exposure to synthetic bat echolocation signals as lek size increased (Mac Nemar, 2-tailed, $G = 0.829$). On the contrary, 72.7% of females (16/22) stopped their evaluation of 2-male leks, whereas only 59.1% (13/22) stopped evaluation of 4-male leks. In the absence of synthetic echolocation signals, all 22 females tested had responded and evaluated both 2-male and 4-male leks.

DISCUSSION

Contrary to some general theoretical predictions (Kirkpatrick 1996; Kirkpatrick and Barton 1997; Cameron et al. 2003; but see Weatherhead and Robertson 1979; Houle and Kondrashov 2002; and Kokko et al. 2006 for other viewpoints), our experiments with *A. grisella* show that females which receive only indirect genetic benefits may incur certain costs during the process of mate choice. We have not determined the absolute cost of searching for and choosing a mate but rather inferred the existence of such expenses indirectly from relative costs: When confronted with an increased number of potential mates, females both lengthen their search trajectories and spend more time evaluating the available males (Figure 3). Thus, females do not exhibit cost-reducing tactics at larger leks but rather make a supplementary expenditure with each additional male that is available to them (but see Gavrilits et al. 2001).

Given that sexual selection theory predicts only minor costs of mate search and choice in cases where females do not

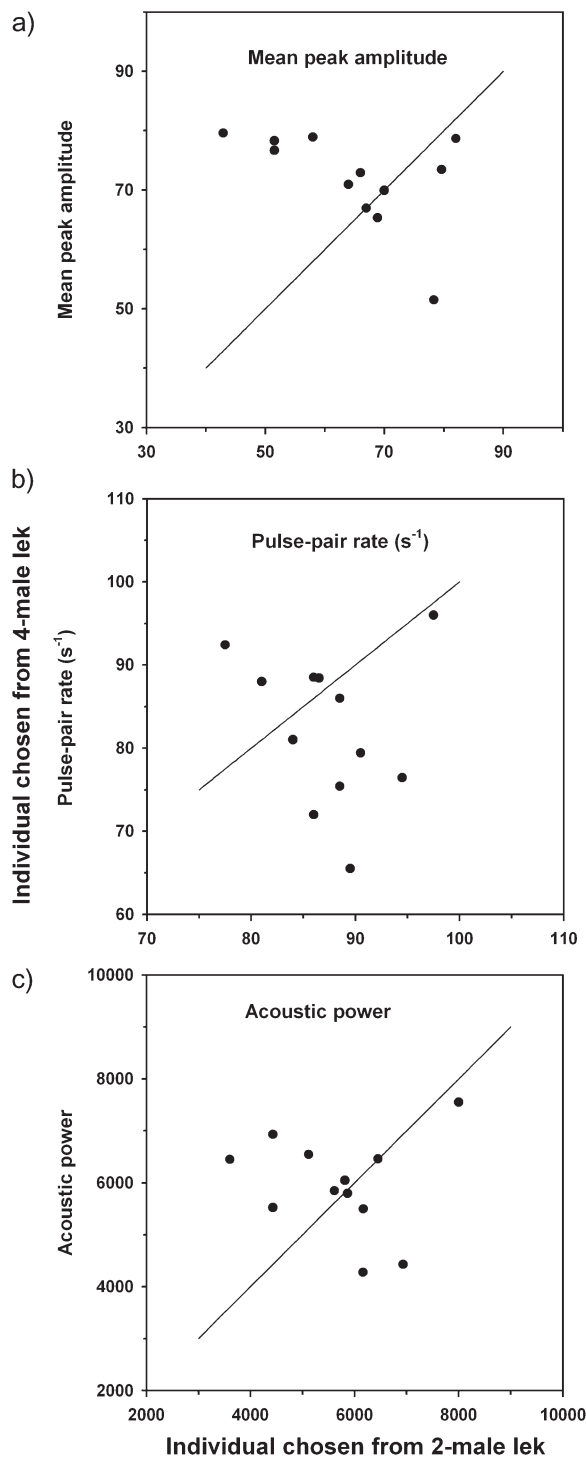


Figure 5

Song characteristics of individuals chosen initially from 2-male leks and second from 4-male leks (experiment 2). Data points represent the 12 females that chose a different male in their second trial, in which a 4-male lek was presented. *x* Axis represents the acoustic character value of the individual chosen from the 2-male lek; *y* axis represents the value of the individual chosen from the 4-male lek. The diagonal line represents equality between the acoustic characters of the 2 chosen individuals; an excess of points above the diagonal would indicate that females chose superior singers from the 4-male lek. (a) Mean peak amplitude, as measured on an arbitrary linear scale (H_0 : acoustic character values of the 2 chosen individuals are equivalent; Wilcoxon signed-rank test, 2-tailed; $W = -0.42$, $P = 0.69$). (b) Pulse-pair rate ($W = 1.10$, $P = 0.30$). (c) Acoustic power, defined as pulse-pair rate \times mean peak amplitude ($W = -0.42$, $P = 0.69$).

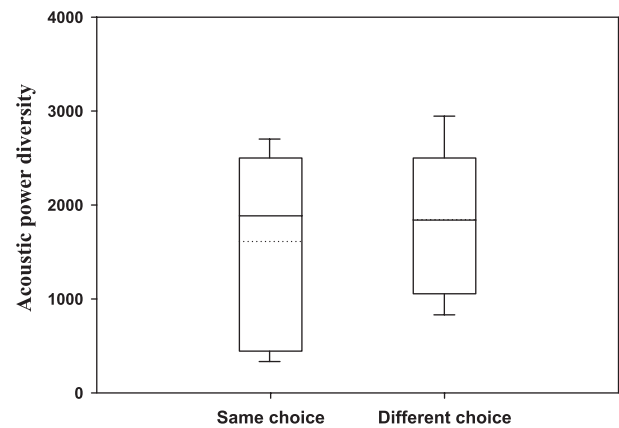


Figure 6

Influence of acoustic power diversity on the consistency of female choice. Box plots indicate mean (dotted line), median (solid line), 25–75% range (box), and 10–90% range (whiskers) of data from 30 female individuals that were presented initially with a 2-male lek and second with a 4-male lek (experiment 2). Left box plot: acoustic power diversity (see text) of 2-male lek presented to females that later chose the same male when presented with the 4-male lek ($n = 18$). Right box plot: acoustic power diversity of 2-male lek presented to females that later chose a different male when presented with the 4-male lek ($n = 12$). (H_0 : power diversity of the 2-male and 4-male leks are equivalent; $t = -0.42$, $P = 0.68$).

receive direct material benefits from males (see Kokko et al. 2006), we consider 3 possible explanations of our findings: 1) the actual costs of mate choice that females in natural populations of *A. grisella* expend are negligible; 2) females do receive direct benefits from mating; or 3) the indirect genetic benefits from mating with a chosen male are considerable. Below, we analyze each possibility in turn, and we conclude that the last, substantial genetic benefits that offset the direct costs of mate choice, is the most likely.

What are the actual costs of mate choice that females in natural populations expend in the process of searching, sampling, and orienting toward a male? We have observed an increased duration and length of the mate sampling trajectory when additional males are present (Figure 3), and it is possible that this trajectory would continue to lengthen somewhat as lek size expands further. However, we propose that the supplementary energy and time spent over the longer distance are negligible, even for a short-lived adult insect that does not replenish its reserves by feeding. On the other hand, females may suffer a genuine risk from predation due to increased exposure during a longer search for leks and, pertinent to the current study, an extended sampling protocol once at a lek. During movement, *A. grisella* would be subject to attack in the air by insectivorous bats (see Rodriguez and Greenfield 2004) and on the ground by nocturnal invertebrate predators such as spiders (Greenfield and Coffelt 1983), and we may infer that the risk of such attack increases linearly with the duration and distance of search. Moreover, depending on the specific location where mate sampling and pairing occurs, *A. grisella* females at a lek site may also risk attacks by insectivorous bats that glean their prey from the substrate. Gleaning bats, which may comprise 30% of the species in various bat faunas (Arlettaz et al. 2001), generally localize their prey by perceiving sound that results from prey activity such as movement or singing. The menace of predation by gleaning bats cannot be overemphasized, particularly in situations where mating activity occurs in open locations outside honeybee colonies: When presented with synthetic bat echolocations

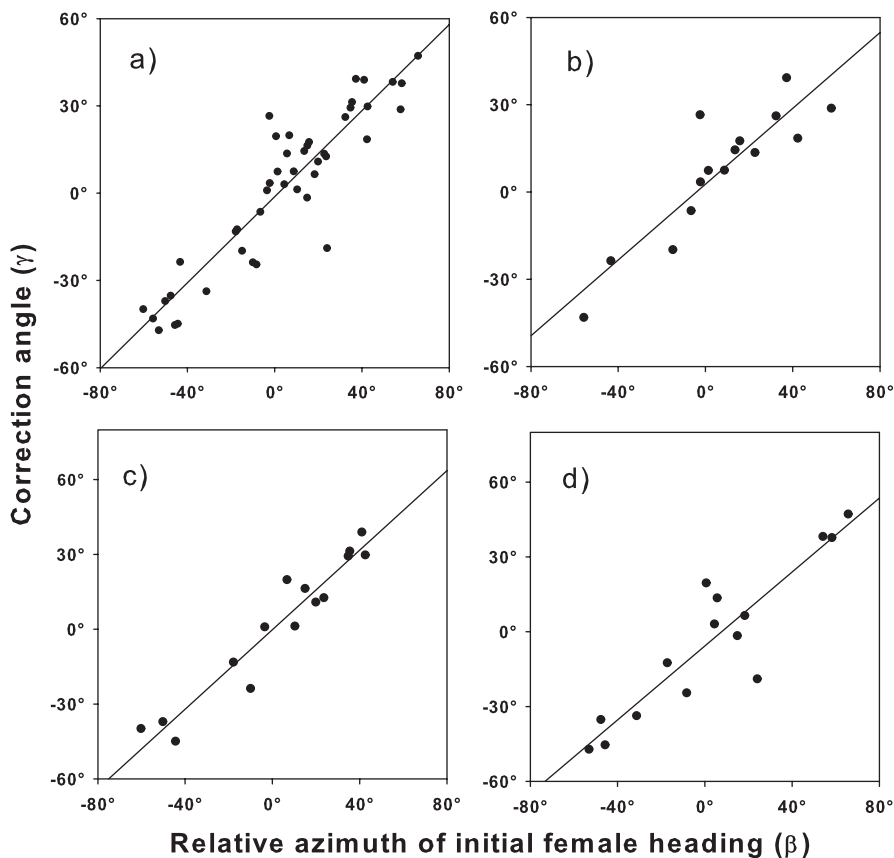


Figure 7

Precision of females orienting toward a single singing male (experiment 3). Scatter plots show average correction angle (γ), made over a 240-ms interval, as a function of average initial heading of female (β ; see text and Figure 2), relative to the male, for 15 females, each measured in 4 separate trials. Lines represent least-squares linear regressions. (a) Data pooled from measurements taken when females were 18-, 12-, and 6-cm distant from the male ($t = 15.06$; $P < 0.001$). (b) Measurements taken 18-cm distant from the male ($t = 7.12$; $P < 0.001$). (c) Measurements taken 12-cm distant from the male ($t = 12.46$; $P < 0.001$). (d) Measurements taken 6-cm distant from the male ($t = 8.09$; $P < 0.001$).

representative of gleaning species, female *A. grisella* typically cease orientation movement toward male song (Greenfield and Weber 2000; Greig and Greenfield 2004), and singing males become silent (Greenfield and Baker 2003). The latter response may indicate that gleaning bats can perceive and then localize male *A. grisella* song or that a singing male risks bat predation if a courted female—which is running, circling, and wing-fanning—is adjacent to him. Thus, we propose that a real cost of extended mate choice does exist and that females pay this cost rather than evaluating only a subset of available males in a larger lek or reducing the thoroughness of their evaluations. We qualify this proposition, though, by noting that under certain conditions, such as when lek size expands markedly or predation pressure becomes stronger, females may no longer pay the cost of increased mate sampling (see Magnhagen 1991). For example, Booksmythe et al. (2008) found that females in the fiddler crab *Uca mjoebergi* reduced their ‘selectivity’ when predation risk increased, even though they received direct benefits at mating.

Our proposition is supported by results from experiment 4, which show that when a predation risk is present, female *A. grisella* are at least as likely to continue their evaluation at larger leks as compared with smaller ones or with solitary males (cf. Greenfield and Weber 2000; Greig and Greenfield 2004). Whereas this result could reflect masking of the echolocation signals by the songs of multiple males or that a female’s greater sexual activation initiated by evaluating multiple males counteracts the inhibitory effect of echolocation signals, the same outcome occurs: A female may pay a cost of evaluation at larger leks by ignoring the danger of predation, a danger which might be higher at larger leks owing to their increased attractiveness to predators.

Assuming that females do incur costs during sampling and choice, we then consider the possibility that real benefits are

forthcoming from their efforts. However, the biology of *A. grisella* suggests that any benefits from choosing one male over another are unlikely to be direct. Males do not provide parental care of any kind, and no observations suggest that critical resources are passed with the spermatophore at mating: Females seldom mate more than once (Greenfield and Coffelt 1983), and females paired with males which have previously mated multiple times do not produce fewer offspring than females paired with males which are mating for the first time (Jia F, unpublished data).

If females are unlikely to receive direct benefits from an extended sampling effort and choice of a mate, are indirect genetic benefits possible? Several lines of evidence indicate yes. First, the heritability of various song characters and of overall song attractiveness, found in various *A. grisella* populations (Collins et al. 1999; Brandt and Greenfield 2004), implies that a choosy female can benefit from her discrimination because she is likely to produce attractive sons. Second, findings from our second experiment show that the level of female choosiness generally remains high as lek size increases. When presented first with a 4-male lek and next with a 2-male lek, 76% of females chose the same male in both trials. Similarly, when presented first with a 2-male lek and next with a 4-male lek, 60% of females chose the same male both times (see Results). We interpret this consistency to indicate that the greater effort in evaluating individuals in the 4-male lek allowed a female to make a specific choice within a larger group. Presumably, the specific choice was based on certain preferred characters of the singing male (see Results for acoustic analysis in experiment 1).

However, when we focused on those females that had not exhibited consistent choices, we did not find evidence that their shifts reflected orientation toward a superior singer that was present only in the 4-male lek (Figure 5). Some of this

inconsistency may reflect a small sample size ($n = 12$): For 8 of 12 females, the male chosen from the 4-male lek sang with greater peak amplitude than the male chosen from the 2-male lek (Figure 5a) (but see Mhatre and Balakrishnan 2008). Other possible explanations of the observed inconsistency in experiment 2 are that individual females vary in their evaluation of male song characters (experiment 1; also see Jang and Greenfield 2000; Rodriguez and Greenfield 2003) and that our understanding of preferred male characters in this *A. grisella* population is incomplete. In a previous study, Jang and Greenfield (1998) found that a multivariate index computed from a selection-gradient analysis of the several acoustic characters measured in male song, including pulse-pair rate and peak amplitude, was more predictive than any single character yet still explained only 63% of the variance in male attractiveness to females (but see RESULTS of experiment 1 for univariate analysis).

We also found no evidence that shifts in choice tended to represent cases where the 2-male lek had included individuals singing with similar acoustic characters, which might have caused the female to choose a male at random. Females which chose a different male from the second, 4-male lek had not necessarily been presented first with a 2-male lek with lower acoustic power diversity than females which made consistent choices had been presented with (Figure 6). More generally, acoustic power diversity or variance did not appear to influence a female's mate sampling protocol (see also Mhatre and Balakrishnan 2007). Rather, her trajectory length, duration, and form could be largely predicted by lek size, indicating that females applied a certain sampling effort to each male and intermale comparison present and that they did so regardless of similarities or differences in the acoustic parameters that we measured. Our analysis of the repeatability of female choice at 4-male leks showed that repeated choices of the same male occurred more often than predicted by the random expectation (Figure 4). These findings suggest that females generally made specific, nonrandom choices of males, and that they expended the sampling effort to retain these choices in more complex situations where they had to evaluate greater numbers of males. Results on the efficiency of acoustic orientation in experiment 3 support this interpretation. Female *A. grisella* oriented with a precision of $\pm 27^\circ$ (Figure 7a), a value comparable with that of observed in various acoustic insects (Greenfield 2002; e.g., see studies on the crickets *Teleogryllus oceanicus* and *Gryllus bimaculatus* by Oldfield 1980 and Rheinlander and Blätgen 1982, respectively). We note that adjacent males in the 4-male leks were separated by an azimuth angle of 90° and that females made many turns that exceeded 45° , and even 90° , while orienting. Given that these turns greatly exceeded the 27° precision, we propose that females would be capable of moving toward a specific male in our arena trials without excessive deviations. The observed inconsistencies in choice may then represent modest levels of confusion by neighboring singers (see Mhatre and Balakrishnan 2008), which could yet occur, as well as aspects of evaluation of male characters that we have not yet identified. Together with the consistency of choice, these latter observations indicate that the greater effort expended by females at larger leks may result more from sampling, particularly for avoiding the most inferior singers (see experiment 1), than from biophysical constraints.

Our analyses show that females may pay the costs of extended mate sampling and choice at leks. These costs might increase commensurately with lek size, but a female can nonetheless benefit by mating with a male that signals in the manner preferred in the population—provided that these signal features are heritable, which is generally the case in *A. grisella*. A costly female choice for indirect genetic benefits as such

may then be sustained at equilibrium, the outcome depending on other costs such as searching for and locating leks. Importantly, we note that these economics may apply even in species in which adult longevity is relatively short, and in which females normally mate but once. Possibly, the critical issue is whether differential sampling costs exist and what their values are relative to genetic benefits (e.g., Head et al. 2005). Conventionally, empirical work on female choice has concentrated on the nature of the benefits that are obtained from mating with a specific male, but a thorough economic analysis would demand attention to the costs as well. Despite the difficulties in assessing these costs, we emphasize that we must continue to explore them if we are to achieve a full understanding of the sexual selection process in natural populations.

FUNDING

Agence Nationale de la Recherche (contrat ANR-07-BLAN-0113-01); Centre National de la Recherche Scientifique; Université François Rabelais de Tours.

We thank Guy Bourdais, Bruno Brizard, and Fabrice Vannier for technical assistance, Jean-Pierre Chartier for helping us to collect our *A. grisella* population. We also thank Marlène Goubault, Michael Jennions, Denis Limousin, Nathan Morehouse, Rafaël L. Rodriguez, and an anonymous referee for valuable criticisms of an earlier version of this manuscript.

REFERENCES

- Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.
- Arlettaz R, Jones G, Racey PA. 2001. Effect of acoustic clutter on prey detection by bats. *Nature*. 414:742–745.
- Bissoondath CJ, Wiklund C. 1996. Male butterfly investment in successive ejaculates in relation to mating system. *Behav Ecol Sociobiol*. 39:285–292.
- Booksmythe I, Detto T, Backwell P. 2008. Female fiddler crabs settle for less: the travel costs of mate choice. *Anim Behav*. 76:1775–1781.
- 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.
- Byers JA, Wiseman PS, Jones L, Roffe TJ. 2005. A large cost of female mate sampling in pronghorn. *Am Nat*. 166:661–668.
- Cameron E, Day T, Rowe L. 2003. Sexual conflict and indirect benefits. *J Evol Biol*. 16:1055–1060.
- Collins RD, Jang Y, Reinhold K, Greenfield MD. 1999. Quantitative genetics of ultrasonic advertisement signalling in the lesser wax moth, *Achroia grisella* (Lepidoptera: Pyralidae). *Heredity*. 83: 644–651.
- Cremer S, Greenfield MD. 1998. Partitioning the components of sexual selection: attractiveness and agonistic behavior in male wax moths, *Achroia grisella* (Lepidoptera: Pyralidae). *Ethology*. 104:1–9.
- Danchin E, Cézilly F. 2005. La sélection sexuelle: un autre processus évolutif. In: Danchin E, Giraldeau LA, Cézilly F, editors. *Ecologie comportementale*. Paris (France): Dunod. p. 235–298.
- Gavriletts S, Arnqvist G, Friberg U. 2001. The evolution of female mate choice by sexual conflict. *Proc R Soc Lond B Biol Sci*. 268:531–539.
- Gibson RM, Bachmann GC. 1992. The costs of female choice in a lekking bird. *Behav Ecol*. 3:300–309.
- Grafen A. 1990. Sexual selection unhandicapped by the Fisher process. *J Theor Biol*. 144:475–1416.
- Greenfield MD. 2002. Signalers and receivers: mechanisms and evolution of arthropod communication. Oxford: Oxford University Press.
- 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 waxmoth, *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.
- Head ML, Hunt J, Jennions M, Brooks R. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol*. 3:289–294.
- Heywood JS. 1989. Sexual selection by the handicap mechanism. *Evolution*. 43:1387–1397.
- Höglund J, Alatalo RV. 1995. *Leks*. Princeton (NJ): Princeton University Press.
- Houle D, Kondrashov AS. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. *Proc R Soc Lond B Biol Sci*. 269:97–104.
- Iwasa Y, Pomiankowski A, Nee S. 1991. The evolution of costly mate preferences. II. The 'handicap' principle. *Evolution*. 45:1431–1442.
- Janetos AC. 1980. Strategies of female mate choice: a theoretical analysis. *Behav Ecol Sociobiol*. 7:107–112.
- 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.
- Jang Y, Greenfield MD. 1996. Ultrasonic communication and sexual selection in waxmoths: 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.
- Jennions MD, Petrie M. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev*. 72: 283–327.
- Jia FY, Greenfield MD. 1997. When are good genes good? Variable outcomes of female choice in wax moths. *Proc R Soc Lond B Biol Sci*. 264:1057–1063.
- Jia FY, Greenfield MD, Collins RD. 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype \times environment interaction. *Evolution*. 54:953–967.
- Kirkpatrick M. 1985. Evolution of female choice and male parental investment in polygamous species: the demise of the sexy son. *Am Nat*. 125:788–810.
- Kirkpatrick M. 1996. Good genes and direct selection in the evolution of mating preferences. *Evolution*. 50:2125–2140.
- Kirkpatrick M, Barton NH. 1997. The strength of indirect selection on female mating preferences. *Proc Natl Acad Sci U S A*. 94:1282–1286.
- Kirkpatrick M, Ryan MJ. 1991. The evolution of mating preferences and the paradox of the lek. *Nature*. 350:33–38.
- Kokko H, Jennions MD, Brooks R. 2006. Unifying and testing models of sexual selection. *Annu Rev Ecol Evol Syst*. 37:43–66.
- Kotiaho JS. 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol Rev*. 76:365–376.
- Kotiaho JS, Puurtinen M. 2007. Mate choice for indirect genetic benefits: scrutiny of the current paradigm. *Funct Ecol*. 21:638–644.
- Künike G. 1930. Zur biologie der kleinen wachsmotte, *Achroia grisella* (Fabricius). *Z Angew Entomol*. 16:304–356.
- Limousin D, Greenfield MD. 2009. Evaluation of amplitude in male song: female waxmoths prefer fortissimo notes. *J Exp Biol*. 212: 4091–4100.
- Magnhagen C. 1991. Predation as a cost of reproduction. *Trends Ecol Evol*. 6:183–186.
- Mhatre N, Balakrishnan R. 2007. Phonotactic walking paths of field crickets in closed-loop conditions and their simulation using a stochastic model. *J Exp Biol*. 210:3661–3676.
- Mhatre N, Balakrishnan R. 2008. Predicting acoustic orientation in complex real-world environments. *J Exp Biol*. 211:2779–2785.
- Oldfield BP. 1980. Accuracy of orientation in female crickets, *Teleogryllus oceanicus* (Gryllidae): dependence on song spectrum. *J Comp Physiol A*. 141:93–99.
- Parker GA. 1978. Searching for mates. In: Krebs JR, Davies NB, editors. *Behavioural ecology: an evolutionary approach*. Oxford (UK): Blackwell. p. 214–224.
- Pomiankowski A, Iwasa Y, Nee S. 1991. The evolution of costly mate preferences. I. Fisher and biased mutation. *Evolution*. 45: 1422–1430.
- Reinhold K, Greenfield MD, Jang Y, Broce A. 1998. Energetic cost of sexual attractiveness: ultrasonic advertisement in waxmoths. *Anim Behav*. 55:905–913.
- Rheinlander J, Blätgen G. 1982. The precision of auditory lateralization in the cricket, *Gryllus bimaculatus*. *Physiol Entomol*. 7:209–218.
- Ritchie MG. 1996. The shape of female mating preferences. *Proc Natl Acad Sci U S A*. 93:14628–14631.
- Rodriguez RL, Greenfield MD. 2003. Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution*. 57:1304–1313.
- 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.
- Spangler HG, Greenfield MD, Takessian A. 1984. Ultrasonic mate calling in the lesser waxmoth. *Physiol Entomol*. 9:87–95.
- Stuart-Fox DM, Moussalli A, Marshall NJ, Owens IPF. 2003. Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Anim Behav*. 66:541–550.
- Weatherhead PJ, Robertson RJ. 1979. Offspring quality and the polygyny threshold: 'the sexy son hypothesis'. *Am Nat*. 113:201–208.
- Wickman PO, Jansson P. 1997. An estimate of female mate searching costs in the lekking butterfly *Coenonympha pamphilus*. *Behav Ecol Sociobiol*. 40:321–328.