

## Energetic cost of sexual attractiveness: ultrasonic advertisement in wax moths

KLAUS REINHOLD\*, MICHAEL D. GREENFIELD\*, YIKWEON JANG\* & ALBERTO BROCE†

\*Department of Entomology, University of Kansas

†Department of Entomology, Kansas State University

(Received 14 November 1996; initial acceptance 5 February 1997;  
final acceptance 16 April 1997; MS. number: A7753R)

**Abstract.** Pair formation in the lesser wax moth, *Achroia grisella* (Lepidoptera: Pyralidae), is initiated by male ultrasonic signals that attract receptive females. Individual males vary in attractiveness to females, and the most attractive males are distinguished by exaggeration of three signal characters: pulse rate, peak amplitude and asynchrony interval (temporal separation between pulses generated by movements of the left and right wings during a given wing upstroke or downstroke). Using flow-through respirometry, we measured the resting and signalling metabolic rates of males whose relative attractiveness was known. Acoustic recordings and metabolic measurements were made simultaneously, and we calculated net metabolic rates and factorial metabolic scopes as measures for the energetic cost of signalling. On average, attractive males had higher net metabolic rates and factorial metabolic scopes than unattractive ones, but many unattractive males also had high values. Thus, high expenditure of energy on signalling is necessary but not sufficient for attractiveness. This may result because only one of the three signal characters critical for female preference, pulse rate, is correlated with energy expenditure. Although the results are consistent with the good genes model of sexual selection, they do not conflict with other indirect or direct mechanisms of female choice.

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It is generally assumed that sexual advertisement signalling by male animals is energetically expensive and risky (Kodric-Brown & Brown 1984; Ryan 1988; Harvey & Bradbury 1991). Measurements of energy expenditure (Vehrencamp et al. 1989; Prestwich 1994; but see Horn et al. 1995) and predation (Cade 1979; Ryan et al. 1982) on signalling males, particularly in acoustic species, support this contention. Because signals vary considerably in many populations and exaggerated levels of certain signal characters typically attract more females (Ryan & Keddy-Hector 1992), the corollary has been proposed that signal attractiveness and energy expenditure are related (Ryan 1988; Gerhardt 1991; Bailey et al. 1993). This proposed relationship forms the basis of the popular hypothesis that characters of advertisement signals are 'reliable' indicators of a male's genetic 'quality', reliability ensured by the inability of

'inferior' males to generate expensive, attractive signals (Kirkpatrick & Ryan 1991; Maynard Smith 1991). Despite its theoretical importance, however, empirical evidence for such a relationship between signal attractiveness and energy is meagre (Taigen & Wells 1985; Wells & Taigen 1989; Watson & Lighton 1994). This critical gap may reflect difficulties in measuring both the energetics of male advertisement signals and their attractiveness.

We directly tested the putative relationship between signal energetics and female attractiveness by studying ultrasonic male advertisements in the lesser wax moth, *Achroia grisella* (Lepidoptera: Pyralidae). Unlike most moths, in which pair formation is initiated by males searching for stationary females emitting olfactory cues (Silberglied 1977), male *A. grisella* produce acoustic signals that attract females up to several metres away (Spangler 1984). These signals are rhythmic pulses produced by stationary, wing-fanning individuals. During wing-fanning, minute tymbals on the tegulae, a pair of sclerites at the bases of

Correspondence and present address: K. Reinhold, Institute for Evolution and Ecology, University of Bonn, An der Immenburg 1, D-53121 Bonn, Germany (email: kreinhold@iaz.uni-bonn.de).

the forewings, are buckled by each upstroke and downstroke; a 100- $\mu$ s pulse of 100-kHz sound is generated at each tymbal buckling (Spangler et al. 1984; Jang & Greenfield 1996). Thus, the pulse rate of the signal reflects the speed of wing movement, an activity expected to be related to energy expenditure (Bailey et al. 1993).

Phonotaxis experiments in the laboratory demonstrate that *A. grisella* males differ greatly in attractiveness to females (Y. Jang & M. Greenfield, unpublished data). Moreover, a male's attractiveness is strongly correlated with values of three signal characters: pulse rate, peak amplitude (loudness) and asynchrony interval (temporal separation between onsets of pulses generated by left and right tymbals during a given wing upstroke or downstroke; see also Jang & Greenfield 1996). Females prefer males signalling with a high pulse rate, high peak amplitude and long asynchrony intervals, but these three signal characters are not correlated (Jang et al. 1997). Peak amplitude has the greatest influence on a male's overall attractiveness, and pulse rate has the least influence. Both overall attractiveness and the three signal characters are highly repeatable within males (Jang et al. 1997).

We evaluated the relative attractiveness of a series of males and then measured their metabolic rates and simultaneously recorded their advertisement signals. Using these measurements, we determined (1) whether attractive males expend more energy and (2) whether exaggeration of any of the three critical signal characters demands higher energy expenditure. The results demonstrate that a relatively high expenditure of energy on signalling is necessary but not sufficient for female attractiveness.

## MATERIALS AND METHODS

### Population Studied

We used a laboratory colony of *A. grisella* derived from animals collected in Auburn, Alabama, four generations prior to the study. Larvae were reared on a diet containing a flour mixture, glycerol, brewer's yeast, beeswax, honey and water (Dutky et al. 1962). Both larvae and adults were kept at 25.5°C on 12:12 h light:dark photoperiod.

To maintain consistency among test animals, all energy and signal measurements were conducted

on virgin adults. We ensured that test animals remained unmated by rearing them individually in 30-ml cups.

### Attractiveness Evaluation

The attractiveness of test males to females was evaluated via phonotaxis trials conducted in a circular screen arena (80 cm diameter) mounted on a turntable. We evaluated four 1-day-old males in a given testing session by placing them individually in cylindrical screen cages (1.5 cm diameter, 2 cm high) situated immediately outside the perimeter of the arena. Azimuthal separations between adjacent cages were 90° relative to the arena centre. The arena was kept in a semi-anechoic room, 3 × 3.5 × 2.5 m, maintained at 25.5°C and illuminated with a 25-W red incandescent bulb. All test males were weighed ( $\pm 0.005$  mg) on a Mettler H51AR balance immediately following their attractiveness evaluation.

We released 20 1-day-old virgin females individually into the arena centre and tallied the number attracted towards each male. A female was judged as attracted towards a male if she moved to within 5 cm of his cage, wing-fanned (a sign of receptivity) and remained there for at least 10 s during a 2-min trial; attraction towards more than one male during a trial never occurred. Each female was tested only once. All trials were conducted during the initial half of scotophase, the period of maximum signalling and mating activity in *A. grisella* (Greenfield & Coffelt 1983). More than 80% of the females used were attracted to a test male.

We monitored the ultrasonic signals emitted by each male with an UltraSound Advice S-25 Bat Detector and released a female only when all four males were signalling. This requirement ensured that males would be evaluated by the quality of their signalling rather than by whether they were undisturbed and remained signalling. The screen of the cages and arena did not interfere with signal transmission (Jang et al. 1997). The arena turntable was rotated 90° after every five trials to minimize potential influences of position effects on attraction of females.

Following testing of all 20 females, we computed each male's attractiveness index as the proportion of responding females that oriented towards him; thus, a male attracting 10 of 20

responding females would have an attractiveness index of 0.5. We repeated the entire attractiveness evaluation procedure 2 days later when the males were 3 days old. We tested 20 sets of four males in this fashion. The attractiveness index of a male was repeatable ( $r=0.55$ ,  $P<0.001$ ) over the two evaluations, and we used the mean of the two values in all analyses. The number of females attracted by individual males deviated from random ( $\chi^2_{60}=447$ ,  $P<0.001$ ).

### Energy Measurements

Because signalling in *A. grisella* is probably an entirely aerobic activity (Prestwich 1994), we used flow-through respirometry to determine the relative metabolic rates of all test males. These relative rates were determined when the males were 2 days old, midway between the two attractiveness evaluations. We calculated resting and signalling metabolic rates by measuring the amounts of CO<sub>2</sub> produced by stationary males during the absence and presence of wing-fanning, respectively. Resting rates were measured 3.5–0.5 h prior to scotophase. Signalling rates were measured 1–4.5 h after the onset of scotophase; signalling (wing-fanning) was confirmed by simultaneous acoustic recording (see Signal Recording). The males were held under their normal photoperiod during the interval between the resting and signalling measurements. Measurements were made at 24°C.

Adult *A. grisella* have an atrophied proboscis and neither feed nor drink. Thus, digestive processes, which can bias the determination of metabolic rate from CO<sub>2</sub> production, do not occur.

CO<sub>2</sub> produced by a resting or signalling male was measured by retaining the insect in a cylindrical screen cage and positioning the cage within a 100-ml glass respirometric chamber. CO<sub>2</sub>-free air was passed through the chamber, and CO<sub>2</sub> concentration in the air exiting the chamber was measured ( $\pm 0.25$  ppm) with an ADC-225-MK3 infra-red gas analyser, calibrated with 0 and 40 ppm reference gases. Flow rate through the chamber was monitored with a Manostat flow meter, calibrated by the bubble method (Levy 1964).

After placing a test male in the respirometric chamber, residual CO<sub>2</sub> was flushed from the chamber with a high flow rate (approximately 400 ml/min) of CO<sub>2</sub>-free air ( $<0.1$  ppm). Once the CO<sub>2</sub> concentration had equilibrated, flow rate was reduced to approximately 45 ml/min. Beginning

8 min after this adjustment, we took 15 readings of the CO<sub>2</sub> concentration at 1-min intervals. Readings were not taken if the concentration changed more than 1 ppm during a 15-min interval, because this indicated that the male was moving or had suddenly stopped (or started) signalling. When these fluctuations occurred, the males were left in the chamber and re-measured only after 15 min of stable CO<sub>2</sub> production was observed.

We calculated the rate of CO<sub>2</sub> production ( $\mu\text{l CO}_2/\text{h}$ ) as the mean of the 15 CO<sub>2</sub> concentrations measured, multiplied by the rate of air flow through the respirometric chamber. To enable comparison with metabolic rates of other species, CO<sub>2</sub> production rates were converted to O<sub>2</sub> consumption rates, assuming a respiratory quotient (ratio of CO<sub>2</sub> production rate to O<sub>2</sub> consumption rate) of 0.71. This respiratory quotient results when animals use fat as the sole metabolic resource (Schmidt-Nielsen 1990). Because adult *A. grisella* neither feed nor drink, their activities probably rely entirely on the metabolism of fat reserves accumulated during larval feeding. Resting and signalling metabolic rates ( $\mu\text{l O}_2/\text{h}$ ) were then corrected to standard temperature and pressure (dry) values. We divided the corrected resting and signalling metabolic rates by male body mass to obtain mass-specific rates ( $\text{ml O}_2/\text{g/h}$ ).

The energetic cost of signalling was calculated as (1) the net metabolic rate, that is the difference between the signalling and resting rates and (2) the factorial metabolic scope, that is signalling rate divided by resting rate. Net metabolic rate represents the absolute energy required to produce the advertisement signals, and factorial metabolic scope represents the factor by which resting metabolic rate must be increased. We report both values, because it is unclear whether one of these represents the cost of signalling better than the other.

### Signal Recording

*Achroia grisella* males readily produce normal signals while kept in small chambers, and we exploited this feature to record their ultrasonic advertisements simultaneously with the energy measurements. A microphone (Brüel & Kjør no. 4138; flat response to 200 kHz) was inserted into a small hole in the respirometric chamber during all measurements of signalling metabolic rate. We lined the inside of the chamber with tissue paper to diminish signal echoes and sealed the junction

between the chamber and microphone to prevent the escape of air. The distance between the microphone and the screen cage retaining the test male was standardized at 4.5 cm to permit comparisons in signal amplitude among insects. The position of the male in the cage influenced the amplitude by less than  $\pm 1.7$  dB, a small value compared to the differences between males.

Signals recorded by the microphone were amplified (Brüel & Kjør preamplifier no. 2633) and transferred to a 486 computer (33 MHz cpu), where they were digitized by a fast analog:digital board (298 kHz sampling rate, well beyond the Nyquist limit). We analysed 120 ms of signalling from each test male and determined pulse rate, mean peak amplitude of the pulses (see caveat concerning mean values in Prestwich 1994) and mean asynchrony interval.

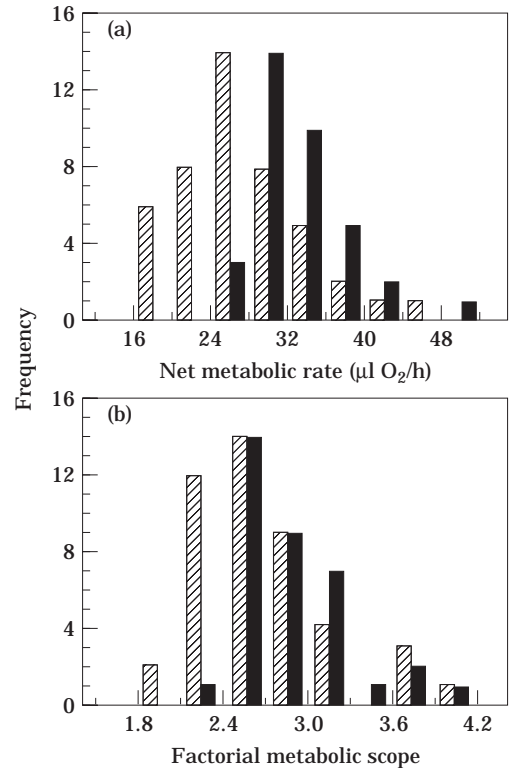
Signals of the test males were also recorded during the trials evaluating attractiveness to females. These recordings, made with a second microphone (ACO Pacifica, Inc. Model 7016; frequency response flat from 10 to 130 000 Hz), allowed us to confirm whether females chose test males based on the various signal characters. By examining the correlations between attractiveness and signal characters and between energy and signal characters, we then determined whether any of the signal characters used by females to choose mates are good predictors of energetic costs.

To determine the energetic efficiency of acoustic signalling (acoustic efficiency), we measured the absolute sound pressure level (0 dB re 20  $\mu$ Pa) of 18 calling males. The sound pressure level of each male was measured at 10 cm and from various angles with a microphone (ACO Model 7016) calibrated with a 16-kHz signal of known amplitude. We converted the sound pressure level values to intensity and then determined acoustic power (nW) by multiplying the average sound intensity by the surface area of a sphere with a 10-cm radius. We calculated acoustic efficiency as acoustic power divided by net metabolic power, the latter value determined via a conversion factor of 1 ml  $O_2$ /h=5.5 mW (Schmidt-Nielsen 1990).

## RESULTS

### Metabolic Rates and Attractiveness

Attractive males (attractiveness index >0.25) incurred higher energetic costs during signalling



**Figure 1.** Energetic cost of signalling given (a) as net metabolic rate and (b) as factorial metabolic scope among attractive (■,  $N=35$ ) and unattractive (▨,  $N=45$ ) *A. grisella* males.

than unattractive males. The net metabolic rates of attractive males ( $N=35$ ,  $\bar{X} \pm SD = 33.3 \pm 5.0$   $\mu$ l  $O_2$ /h) averaged 23% greater than the rates of unattractive males ( $N=45$ ,  $27.1 \pm 6.4$   $\mu$ l  $O_2$ /h; Mann-Whitney  $U$ -test:  $U=1257$ ,  $N_1=35$ ,  $N_2=45$ ,  $P<0.001$ ; Fig. 1a). Similarly, factorial metabolic scopes of attractive males exceeded those of unattractive males ( $U=1024$ ,  $N_1=35$ ,  $N_2=45$ ,  $P<0.05$ , Fig. 1b). Although the difference in net metabolic rate might reflect greater expenses intrinsic to the signalling activity of attractive males, the difference could also result from a disparity in size. Because attractive males ( $N=35$ ,  $13.8 \pm 1.9$  mg) were approximately 10% heavier than unattractive ones ( $N=45$ ,  $12.6 \pm 1.7$  mg,  $U=1084.5$ ,  $P<0.005$ ) and net metabolic rate was positively correlated with body mass ( $N=108$ ,  $r=0.482$ ,  $P<0.01$ ), the observed relationship between net metabolic rate and attractiveness could reflect merely the greater

body mass of attractive males. To eliminate this possibility, we examined the residuals of the net metabolic rate versus body mass regression. These residuals, by definition uncorrelated with body mass, are significantly higher for attractive than unattractive males ( $U=1165$ ,  $N_1=35$ ,  $N_2=45$ ,  $P<0.001$ ). Therefore, we infer that the activities associated with attractive signalling incur a greater energetic cost.

Although attractive males incurred significantly higher energetic costs on average, several unattractive males had net metabolic rates as high as the most attractive males (Fig. 1a). This scatter among the data could indicate that some features of attractiveness are not related to signal energetics. Alternatively, the scatter may result from inaccurate classification of males into the attractive and unattractive categories. That is, our classification was based on a male's attractiveness among a group of four randomly selected males. Consequently, some males from among the attractive half of the population at large may have been ranked as unattractive because they were by chance paired with three more attractive males. To eliminate this possible interference in the relationship between attractiveness and energetics, we re-analysed the data by retaining within-group rankings as follows. Within each of the 20 groups of four males evaluated for attractiveness to females, we ranked males according to their attractiveness index, their mass-specific net metabolic rate, and their factorial metabolic scope. The ranks for attractiveness index and mass-specific net metabolic rate were positively correlated in a significant proportion of the groups (sign test:  $P<0.01$ ; Table I; Fig. 2a). We also found an overall positive correlation between attractiveness index and factorial metabolic scope (sign test:  $P=0.02$ ; Table I; Fig. 2b). In both sets of correlations, however, some males were still ranked high in energetic costs but low in attractiveness. Thus, signalling energetics account for some, but not all, of the variation in male attractiveness. We used mass-specific net metabolic rate and factorial metabolic scope in the above analysis, because neither of these parameters were positively correlated with body mass (mass specific net metabolic rate:  $N=108$ ,  $r=-0.22$ ,  $P=0.02$ ; factorial metabolic scope:  $N=108$ ,  $r=-0.07$ ,  $P=0.40$ ). Therefore, these relationships between attractiveness and energetic cost rankings cannot be confounded by body mass.

**Table I.** Relationship between a male's relative attractiveness and features of his signalling (mass-specific net metabolic rate, net metabolic rate, factorial metabolic scope and rate) within each of 20 four-male groups (80 males total)

Signalling feature	$s>0$	$s=0$	$s<0$
Mass specific net metabolic rate*	16	0	4
Net metabolic rate**	17	1	2
Factorial metabolic scope*	14	2	4
Pulse rate*	15	1	4

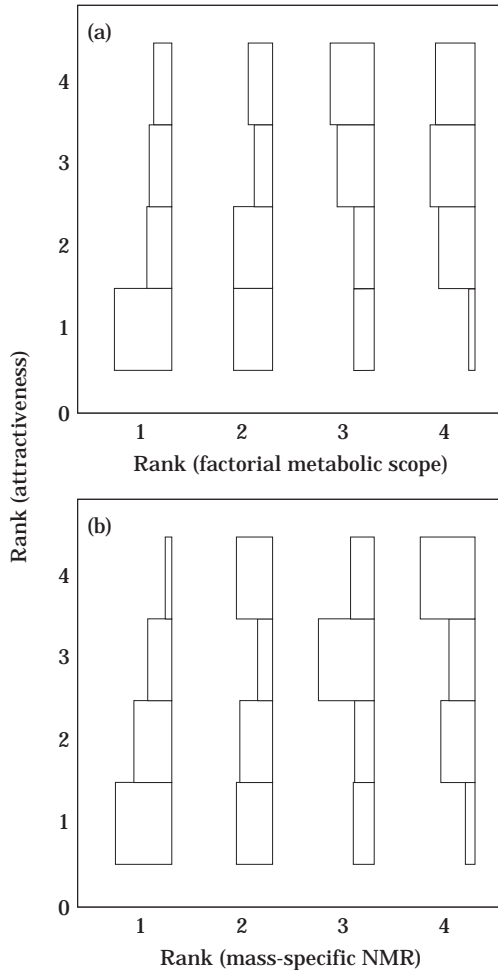
Asterisks denote significant, positive association between the signalling feature and attractiveness; two-tailed sign test. \* $P<0.05$ ; \*\* $P<0.001$ .

Within each group, Spearman rank correlations between signalling feature and attractiveness were determined. Values represent the number of groups with positive ( $>0$ ), 0 and negative ( $<0$ ) correlation.

### Metabolic Rates and Signal Characters

Pulse rate, which directly reflects wingstroke rate, was significantly correlated with net metabolic rate ( $N=108$ ,  $r=0.68$ ,  $P<0.001$ ; Fig. 3a); mean peak amplitude ( $r=0.13$ ,  $0.18$ ,  $P>0.1$ ; Fig. 3b) and mean asynchrony interval ( $r=0.04$ ,  $0.12$ ,  $P>0.2$ ; Fig. 3c) were not. This was corroborated by step-wise multiple correlation analysis, which showed that adding mean peak amplitude or asynchrony interval as predictor variables for net metabolic rate did not significantly improve the correlation achieved with pulse rate as the sole predictor ( $P>0.1$  for both characters). Pulse rate was also significantly correlated with factorial metabolic scope ( $N=108$ ,  $r=0.48$ ,  $P<0.001$ ), and, as with net metabolic rate, mean peak amplitude ( $r=0.18$ ,  $P>0.1$ ) and mean asynchrony interval ( $r=0.12$ ,  $P>0.2$ ) were not significantly correlated with factorial metabolic scope.

Recordings made during the trials evaluating male attractiveness confirmed that females chose test males based, in part, on pulse rate. As above, within each of the 20 groups of four males, we ranked males according to their attractiveness index and their pulse rate. These ranks were positively correlated in a significant proportion (16 of 20, sign test:  $P<0.01$ ; Table I; Fig. 4) of the groups. Again, scattering of the ranks was evident, because some males were ranked high in pulse rate but low in attractiveness.



**Figure 2.** Relationship between a male's attractiveness and his energetic cost of signalling within the 20 four-male groups (80 males total). Within each group, ranks are arranged from lowest (1, smallest energetic cost or lowest attractiveness) to highest (4). Each of the four vertical histograms shows the distribution among attractiveness ranks of the 20 males of a given energy rank. (a) Energetic cost of signalling measured as mass-specific net metabolic rate (NMR). (b) Energetic cost of signalling measured as factorial metabolic scope.

### Acoustic Energetics and Efficiency

The mean  $\pm$  SD basal and net metabolic rates of the *A. grisella* males tested were  $17.6 \pm 3.0 \mu\text{l O}_2/\text{h}$  ( $N=108$ ) and  $30.2 \pm 6.6 \mu\text{l O}_2/\text{h}$ , respectively. Mass of the tested males averaged  $13.1 \pm 1.8 \text{ mg}$ , yielding mean mass-specific basal and net metabolic rates of  $1.34 \text{ ml O}_2/\text{g/h}$  and  $2.32 \text{ ml O}_2/\text{g/h}$ ,

respectively. The mean factorial metabolic scope was  $2.75 \pm 0.43$  ( $N=108$ ). Determined from the ratio between average net metabolic rate and average pulse rate (42.6/s), a single wingstroke costs approximately  $0.20 \text{ nl O}_2$ .

Net metabolic rate was not correlated ( $r = -0.11$ ,  $P > 0.2$ ) with the diel time of measurement. That is, although our procedure for determining metabolic rates was lengthy and led to some males being measured 3 h later during photophase (or scotophase) than others on a given day, this had no influence on energy consumption.

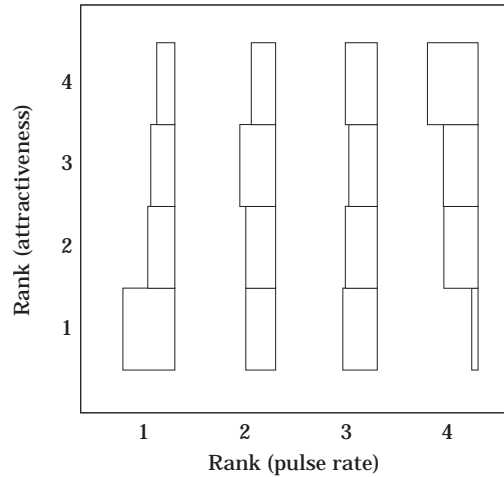
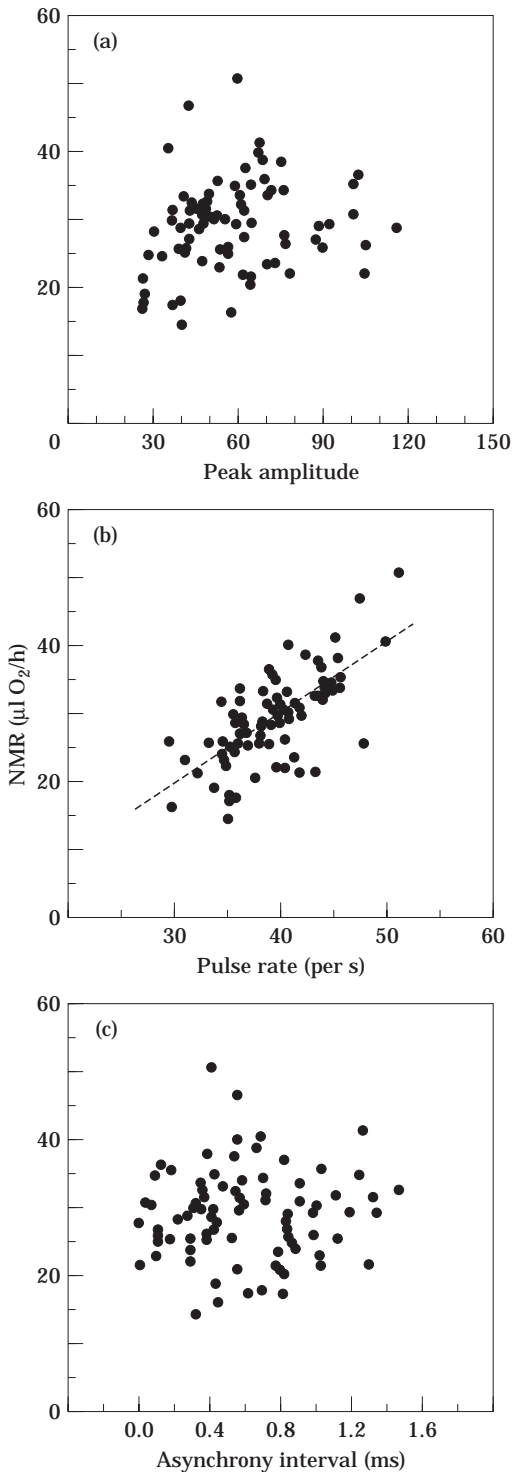
Test males lost mass at an average rate of  $0.82 \pm 0.20 \text{ mg day}$  ( $N=93$ ) over the 2-day measurement period. Assuming that males signal and rest for approximately 8 and 16 h per day (K. Reinhold, unpublished observations), respectively, mean long-term  $\text{O}_2$  consumption was estimated at  $0.66 \pm 0.10 \text{ ml day}$  ( $N=108$ ) or  $0.95 \text{ mg day}$ . Estimated daily  $\text{O}_2$  consumption and mass loss were significantly correlated ( $N=93$ ,  $r=0.51$ ,  $P < 0.001$ ).

Mean intensity of the moths' signals was 50 dB at a distance of 10 cm, yielding a mean acoustic power output of  $12 \pm 4 \text{ nW}$ . Mean net metabolic power was  $0.17 \text{ mW}$  ( $5.5 \text{ mW/ml O}_2/\text{h}$  times  $30.25 \mu\text{l O}_2/\text{h}$ ), and the mean acoustic efficiency was therefore 0.008%.

## DISCUSSION

### Female Choice, Signal Characters and Signal Energetics

*Achroia grisella* females choose males based on the pulse rate of their ultrasonic advertisement signals, as well as several other signal characters. Because pulse rate is a good predictor of net metabolic rate, female choice leads to the selection of males that incur increased energetic costs during signalling. Some of the increased costs incurred by attractive males can be explained simply by the correlations between male size and attractiveness and between male size and net metabolic rate; that is, females choose larger males, which have higher net metabolic rates by virtue of their size alone. Analysis of the residuals in the net metabolic rate versus size regression, however, indicates that attractiveness per se demands increased energetic cost.



**Figure 4.** Relationship between a male's pulse rate and his attractiveness within each of the 20 four-male groups used for attractiveness evaluation (80 males total). Within each group, ranks are arranged from lowest (1, slowest pulse rate or lowest attractiveness) to highest (4). Each of the four vertical histograms shows the distribution among attractiveness ranks of the 20 males of a given rank for pulse rate.

Although all attractive males incurred relatively high energetic costs during signalling, not all males that incurred such costs were attractive (Figs 1 and 2). Thus, energetic expenditure on signalling is necessary but not sufficient for attractiveness. This relationship probably reflects the correlation between net metabolic rate and only one of the signal characters, pulse rate, that influence overall attractiveness. Pulse rate of the signal is identical to the insect's wingstroke rate and the contraction rate of its indirect flight muscles. Such rates are expected to be positively related to energy expenditure (Bailey et al. 1993). Peak amplitude and asynchrony interval, however, may be influenced largely by morphology and neural activity, respectively. It is not clear how energy expenditure could be directly related to these signal characters.

**Figure 3.** Relationship between net metabolic rate and signal characters in *A. grisella*: (a) net metabolic rate versus mean peak amplitude (units in a linear relative scale); (b) net metabolic rates versus pulse rate; (c) net metabolic rates versus mean asynchrony interval. See text for definitions of signal characters.

### Signalling Energetics and Efficiency among Acoustic Insects

The factorial metabolic scope of signalling in *A. grisella* males, 2.75, is the lowest reported in any acoustic insect that produces a continuous, trilling call (Bailey et al. 1993; Prestwich 1994). This low value may be related to the relatively high resting metabolic rate (1.34 ml O<sub>2</sub>/g/h; see Bailey et al. 1993), the lack of adult feeding and the prolonged diel signalling period (6–10 h). A high mass-specific resting metabolic rate is generally associated with small size (Withers 1992), and the mass of *A. grisella* males is only 25% that of the lightest acoustic animal previously measured (Table 1 in Prestwich 1994). The energy that fuels signalling in *A. grisella* is derived entirely from food reserves acquired during the larval period. These reserves are not replenished during the 7–14-day period of adult signalling (longevity), and a signalling male loses more than 5% of his body mass daily. Consequently, selection may favour a signalling mechanism that incurs little increase in energetic cost over basal metabolism. The prolonged diel period of signalling in *A. grisella*, which may be influenced by the insect's mating system, may also select for a mechanism incurring little energetic cost. This possibility is especially likely, given the limited energy available to adults.

The acoustic efficiency of *A. grisella* signals is also lower than that reported in any other acoustic insect (Prestwich 1994), which may reflect the low duty cycle (approximately 0.02) of the signal as well as the insect's small size and high sound frequency.

### Sexual Selection and Costly Signals

Does the association between female attractiveness and net metabolic rate during signalling (Figs 1 and 2) allow us to identify the mechanism of sexual selection in *A. grisella*? Because attractive males bear higher net energy costs, one might infer that females choose (genetically) viable males capable of sustaining such costs and that a good genes mechanism (Grafen 1990) operates. Additional considerations, however, demonstrate that such inference would be premature.

First, attractive males on average do show higher net metabolic rates, but the rates of some unattractive males are comparably high (Figs 1

and 2). Thus, high energy expenditure is necessary but not sufficient for attractiveness, a conclusion substantiated by our observation that female choice is based on non-energetic signal characters (peak amplitude, asynchrony interval) as well as an energetic one (pulse rate). None the less, exaggeration of non-energetic signal characters could depend on energetic costs sustained earlier during larval growth and which yield specific development of the tegular tymbal device, so this consideration does not necessarily rule out a good genes mechanism.

Second and more importantly, our findings on *A. grisella* are not inconsistent with other indirect and direct sexual selection mechanisms (Balmford & Read 1991). Female choice for males producing costly signals can also result from arbitrary (Fisherian) selection, or it might represent a means of obtaining direct benefits such as a parasite-free mate or ease in mate localization.

Thus, measurements of signalling energetics, although adding a critical dimension to our understanding of a sexual selection system, cannot identify the specific mechanism(s) that operate. Definitive answers to this central question will be forthcoming only from carefully designed breeding experiments and quantitative genetic analyses (Jia & Greenfield 1997).

### ACKNOWLEDGMENTS

We are grateful to Robert Minckley for collecting wax moths, to T. Ohno for constructing the respirometric chamber, to Ken Hampton for advice on operating the infra-red CO<sub>2</sub> analyzer, and to Craig Martin for flowmeter calibration. The assistance of Jamie McCullough, Nathan Orr and Feng-You Jia with insect rearing, attractiveness evaluation and signal recording was indispensable. Tom Peters and Rick Roggero, both of the University of Kansas Instrumentation Development Laboratory, developed custom computer software for ultrasonic signal acquisition and analysis. The manuscript benefited greatly from reviews by Bob Collins, Klaus-Gerhard Heller, Andy Snedden, Ken Prestwich and several anonymous referees. This work was funded by U.S. National Science Foundation grant IBN 9407162 to M.G. and DFG grant RE 1167/1-1 to K.R.

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