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ARTICLES

Acoustics, context and function of vibrational signalling in a lycaenid butterfly–ant mutualism

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Juveniles of the Australian common imperial blue butterfly, *Jalmenus evagoras,* produce substrate-borne vibrational signals in the form of two kinds of pupal calls and three larval calls. Pupae stridulate in the presence of conspecific larvae, when attended by an ant guard, and as a reaction against perturbation. Using pupal pairs in which one member was experimentally muted, pupal calls were shown to be important in ant attraction and the maintenance of an ant guard. A pupa may use calls to regulate levels of its attendant ants and to signal its potential value in these mutualistic interactions. Therefore substrate-borne vibrations play a significant role in the communication between *J. evagoras* and its attendant ants and pupal calls appear to be more than just signals acting as a predator deterrent. Similarly, caterpillars make more sound when attended by *Iridomyrmex anceps*, suggesting that larval calls may be important in mediating ant symbioses. One larval call has the same mean dominant frequency, pulse rate, bandwidth and pulse length as the primary signal of a pupa, suggesting a similarity in function.

From the foot-drumming of a banner-tailed kangaroo rat, *Dipodomys spectabilis*, in the presence of a snake (Randall & Matocq 1997) to the coordinated group chorusing of nymphal treehoppers (Cocroft 1996), vibrational signalling is a widespread form of communication, functioning primarily in defence, mate attraction and displays of aggression. Instances of such communication between unrelated species, however, are relatively rare. Nevertheless, recent work indicates that vibrational communication may play a vital role in butterfly–ant mutualisms, wherein caterpillars and pupae use an intricate combination of chemical, behavioural and secretory signals to maintain a retinue of ants that protect them from predators and parasites. DeVries (1990) showed that caterpillars that interact with ants are capable of producing vibratory 'songs'. These larvae are found exclusively in the butterfly families Lycaenidae and Riodinidae. In comparing ant attendance levels between control larvae of the riodinid *Thisbe irenea* and larvae that had been experimentally muted, calling *T. irenea* caterpillars were tended by more ants, indicating that one function of riodinid calls is ant attraction. DeVries concluded: 'under selection for symbiotic associations, the calls of one insect species have evolved to attract other, distantly related insect species' (DeVries 1990, page 1106).

Both larvae and pupae of some species of Lycaenidae can produce sound (Dodd 1916; Downey 1966; DeVries 1990). Larvae produce vibrations that are primarily substrate borne, although they may have a slight airborne component (DeVries 1991a; M. Travassos, personal observation), whereas pupae produce signals with both vibrational and airborne components (Hoegh-Guldberg 1972; Downey & Allyn 1978). In pupae, a file of teeth on the anterior side of the sixth abdominal segment grates against an opposing plate on the posterior side of the fifth abdominal segment (Downey 1966). Such a plate may be made up of either tubercles, reticulations, or ridges. The mechanism of sound production in lycaenid larvae, however, has proved more elusive. Hill (1993) proposed a possible stridulatory organ similar to that found in pupae: a file of teeth and an opposing plate. Caterpillars in the family Riodinidae, the sister taxon to the Lycaenidae (Kristensen 1976; D. Campbell, A. Brower, N. Pierce, unpublished data), signal by beating vibratory papillae against epicranial granulations; lycaenids lack such a structure (e.g. Cottrell 1984).

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At least half of all lycaenids interact with ants, varying from facultative interactions where juveniles are found with or without ants, and often with many species, to obligate ones where juveniles cannot live without ants and usually associate with only one or two closely related species (Pierce 1987; Fiedler 1991). Not only are myrmecophilous lycaenids protected against ants themselves, which might otherwise be threatening predators (Malicky 1970), but it has been shown experimentally that attendant ants also protect juveniles from predators and parasites (e.g. Pierce et al. 1987; Fiedler & Maschwitz 1988a; DeVries 1991c; Cushman et al. 1994; Wagner 1994). Dodd (1916) and DeVries (1990) suggested that lycaenid caterpillar calls, which are essentially substrate-borne vibrations, may be important in mediating ant interactions, because ants, although nearly deaf to airborne sounds, are sensitive to vibration (Fielde & Parker 1904; Hölldobler & Wilson 1990). Lycaenid larvae are able to produce several different vibrational signals. DeVries (1991a) noted that some lycaenid sounds have two components: a low background sound accompanied by a louder pulsing. *Leptotes cassius*, for example, has 'a ticking background and an irregular, galloping series of trills' (DeVries 1991a, page 17). No experimental work has examined the function of lycaenid larval calls.

Research on the function of pupal calls has been inconclusive. Functional explanations for lycaenid pupal signals have speculated about their role in defence (Hinton 1948; Downey & Allyn 1978), conspecific attraction in the formation of aggregations (Prell 1913), and myrmecophily (Downey 1966; Elfferich 1988; Brakefield et al. 1992). The acoustic characteristics of such signals are better known. Lycaenid pupae produce three distinct signals, each distinguishable by amplitude level (Downey & Allyn 1978). The loudest pulse, the primary signal, is also the longest. Secondary signals are briefer and are often found in pulse trains. Tertiary signals have only been recorded in large pupae and consist of irregular click trains not much louder than background noise. Although Downey (1966) and Hoegh-Guldberg (1972) reported a correlation between primary signal production and movement between the fifth and sixth abdominal segments, no observations of abdominal movement have been made regarding the production of secondary and tertiary signals.

We analysed the acoustic properties, context and function of sound production in juveniles of the Australian lycaenid *Jalmenus evagoras*. Larval and pupal *J. evagoras* associate with ants of several species in the genus *Iridomyrmex*. In return for producing nutritious secretions, caterpillars and pupae receive protection against predators and parasites (Pierce et al. 1987). *Jalmenus evagoras* larvae have several mechanisms to attract and appease attendant ants (reviewed in Kitching 1983), including a dorsal nectary organ (DNO) on the seventh abdominal segment that produces a sugary secretion that the ants imbibe, and surface epidermal glands called perforated cupola organs (PCOs) scattered along the length of the cuticle that are thought to produce substances important in both appeasement and reward. PCOs are also found on pupae, which lack a functional DNO. On the eighth abdominal segment, larvae have a pair of eversible tentacle organs (TOs), believed to release a volatile chemical that alerts attendant ants if a larva is alarmed or the DNO is depleted (Henning 1983; Fiedler & Maschwitz 1988b).

Late-instar larvae and pupae of *J. evagoras* stridulate when disturbed (Pierce et al. 1987). Like other lycaenids (Downey 1966), pupae have a file-and-plate stridulatory organ between the fifth and sixth abdominal segment. The pupal plate extends the length of the intersegmental region and has a series of ridges against which the teeth grate. DeVries (1991a, page 17) found that pupae produce 7.5 'metallic click-like pulses' per second, each with a frequency of 2300 Hz, and that larvae of *J. evagoras* produce a drumming call resembling a 'khen-khen-khen-khen' at a rate of 7 calls/s and a mean frequency of 1700 Hz. Our study aimed to investigate the parameters of these calls in greater detail and to explore their functional significance. We focus on the vibrational components of these sounds, measured with an accelerometer. Throughout, except where noted otherwise, we use the terms 'sounds' and 'calls' for simplicity in describing these substrate-borne vibrations. Nothing is known of the sensitivity of lycaenid larvae and pupae to airborne versus substrate-borne signals; ants are nearly deaf to airborne sound, but sensitive to vibrations (Fielde & Parker 1904; Hölldobler & Wilson 1990).

GENERAL METHODS

The study was conducted in the Museum of Comparative Zoology Laboratories from June 1996 to February 1997. We collected *J. evagoras* eggs from field sites in Ebor, New South Wales (30°24′S, 152°19′E), Mount Nebo, Queensland (27°24′S, 152°47′E), and Canberra, Australian Capital Territory (35°21′S, 148°56′E), Australia. Queenright colonies of *Iridomyrmex anceps* maintained in the laboratory were collected from Mount Nebo, Canberra, and Griffith, Australia (27°33′S, 153°3′E). Ant colonies were fed an artificial diet (Bhatkar & Whitcomb 1970) and chopped crickets daily. Larvae of *J. evagoras* were reared on *Acacia melanoxylon* and *A. irrorata* raised from seed purchased from the Queensland Forestry Department.

All calls were recorded in an experimental arena measuring 132 × 66 cm and 132 cm high with two vertical surfaces covered in black construction paper to reduce the effects of external stimuli such as sunlight. To allow for manipulation of the set-up, the other two vertical surfaces remained uncovered. The room was maintained at a constant temperature of 22°C and had fluorescent lighting overhead.

Because of their low amplitude, lycaenid calls are difficult to analyse. Although Hoegh-Guldberg (1972) concluded that there was no resonance from using recording vials to amplify airborne signals, Downey & Allyn (1978) showed that this method, and the use of a directional microphone, introduces artefacts such as standing waves and frequencies. DeVries (1991b) used a particle velocity microphone and amplifier attached to a paper or mylar membrane that acted as a recording stage. He placed larvae or pupae on the membrane and recorded their substrate-borne vibrations.

Table 1. Call characteristics for each juvenile call (mean±SE)

Call	Frequency (Hz)	Bandwidth (Hz)	Pulses/s	Pulse length (s)	Relative amplitude (dB)
Pupae					
Primary signal	849.2±31.0	1435.4±62.8	1.76±0.23	0.082±0.008	Standard
Secondary signal	772.6±90.7	1098.3±170.8	9.24±2.54	0.033±0.004	−5.9±1.2
Larvae					
Grunt	754.4±34.6	1361.4±75.0	2.01±0.36	0.106±0.018	Standard
Drum	471.7±79.5	831.7±129.4	8.29±0.33	0.040±0.005	−2.7±0.9
Hiss	444.1±39.2	778.2±31.6	6.39±0.47	0.050±0.007	−9.8±1.5

We used a different approach. We recorded the vibrations using two accelerometers (BU-3170 and BU-1771, Knowles Electronics Inc., Itasca, Illinois). These accelerometers have sensitivity ranges of 20–3000 and 50–3000 Hz, respectively. Downey & Allyn (1978) determined that pupal calls fall between 400 Hz and 5000 Hz and DeVries (1991a) found that lycaenid caterpillar calls have a mean frequency of about 1 kHz. Initial tests indicated that pupal calls of *J. evagoras* fell within the lower half of this range. Each accelerometer weighed 0.28 g, and measured 7.92 × 5.59 × 4.14 mm. Recorded vibrations were amplified with an Archer Mini-Amplifier and recorded on a Nagra IV-SJ tape recorder. We amplified the calls further on the tape recorder by 40 dB. One channel recorded vibrational signals from the accelerometer, the other spoken behavioural observations. For the experiments we placed larvae on *Acacia* branches that were 1–5 mm in diameter. An accelerometer was firmly taped to the branch so that it was in close contact with the plant surface and oriented so that its axis of acceleration was normal to the plant surface. For experiments with pupae, an accelerometer was firmly taped to a wooden stick on which a caterpillar had pupated, its axis of acceleration normal to the stick's surface.

CALL ANALYSIS

Methods

We examined the context of larval and pupal calls (see below) and then used the samples of vibrational signals obtained from these experiments to analyse the acoustic repertoire of *J. evagoras*. We examined these samples with Canary 1.2b 1994, a sound analysis program produced by the Cornell Laboratory of Ornithology. We defined the beginning and end of a call with respect to the background noise level. To control for differences in recording quality and degree of filtering, we used a uniform brightness and contrast setting for all spectrograms. However, the signal-to-noise ratio may not have been completely comparable for all recordings used. For each call, we measured four properties. We calculated the dominant frequency as the average of the upper and lower frequency bounds of a call. The bandwidth of a call consisted of the difference between these upper and lower bounds. We measured the pulse length as the duration of a call, and we calculated the pulse rate, measured only for calls in pulse trains, as the inverse of the time until the next pulse was produced. All four characteristics were measured for each call sample. For each subject, we averaged the parameter values for each call. The call characteristics of 11 pupae were derived from a total of 108 calls, while the call characteristics of nine larvae were calculated from 125 calls.

To determine the relative amplitudes of a pair of calls, we used recordings of a subject producing both call types in the same trial. Whenever possible, we made 10 peak-to-peak voltage measurements for each call type and then averaged these for each subject. Amplitude differences between a subject's different calls were measured in decibels. The relative amplitudes of the calls of 10 pupae were derived from a total of 144 signals, while the relative amplitudes of the calls of 13 larvae were calculated from 294 signals.

Counts given with each call refer to the number of subjects sampled for each particular call type. We performed pooled comparisons between parameters of different calls with Mann–Whitney *U* tests. Comparisons between the peak-to-peak voltages of pairs of different calls were made with Wilcoxon signed-ranks tests. In determining significance, ties were taken into account. We calculated relationships between pairs of variables as Pearson's correlations.

Results

Pupae of *J. evagoras* produced two types of substrate-borne vibrations (Table 1), which matched Downey & Allyn's (1978) description of primary and secondary signals; no tertiary signals were detected. Primary signals (N=11) had a higher amplitude than secondary signals (N=5; Wilcoxon test: T=55, N=10, P<0.01), which were typically found interspersed between primary signals (Fig. 1a) or in pulse trains. Both kinds of signals were also produced as single pulses. They did not differ significantly in mean dominant frequency (Mann–Whitney U test: U=22.00, N_1=11, N_2=5, NS) or bandwidth (U=13.00, N_1=11, N_2=5, NS).

Larvae produced three kinds of calls. The call with the highest amplitude sounded like a grunt (N=9) and had the longest pulse length and the highest mean dominant frequency of the three larval calls (Fig. 1b). *Jalmenus evagoras* caterpillars also produced a lower-amplitude, lower-frequency call (N=6) resembling the sound of a cat

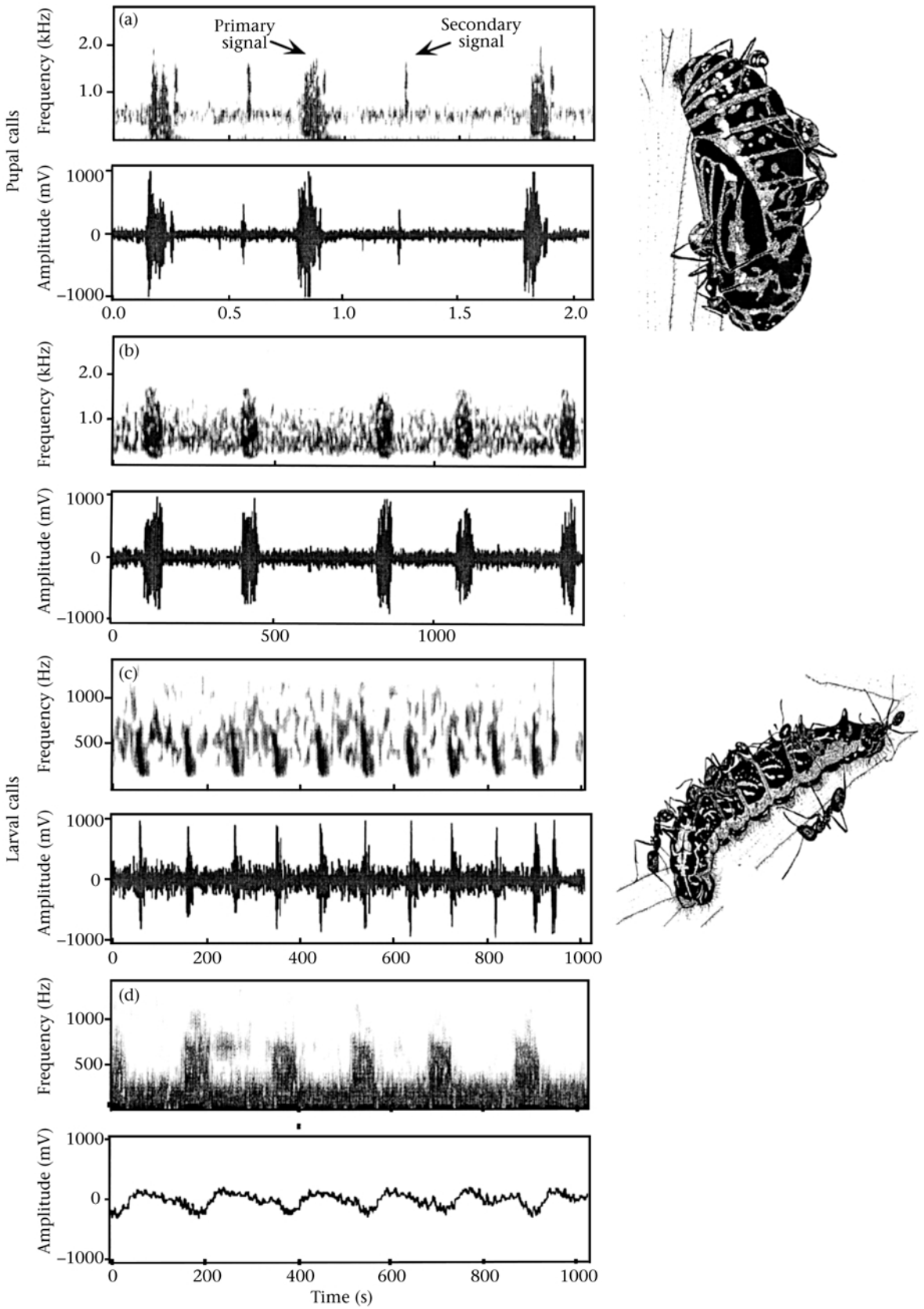

Figure 1. Spectrogram (top) and waveform (bottom) of (a) the primary and secondary signals produced by *J. evagoras* pupae, and a train of (b) grunts, (c) drums and (d) hisses produced by *J. evagoras* larvae. Drawings by Christopher Adams.

purring or a low-pitched drumming (Fig. 1c). The lowest-amplitude call (N=3) sounded like a rapid 'hiss-hiss-hiss' and, like the drum call, was found only in pulse trains (Fig. 1d). There was no significant difference between the mean dominant frequency (Mann–Whitney U test: U=7.00, N_1=6, N_2=3, NS), pulse length (U=4.50, N_1=6, N_2=3, NS), or bandwidth (U=9.00, N_1=6, N_2=3, NS) of the drum and hiss calls. The drum call, however, had a higher pulse rate (U=0.00, N_1=6, N_2=2, P<0.05) and was 5.9 dB louder than the hiss call (N=8), a significant difference (Wilcoxon test: T=28, N=7, P<0.05). Both of these calls differed significantly from the grunt with respect to all four parameters.

The larval grunt call and the pupal primary signal were remarkably similar. There was no significant difference between them in mean dominant frequency (Mann–Whitney U test: U=25, N_1=9, N_2=11, NS), pulse rate (U=25, N_1=7, N_2=9, NS), pulse length (U=15, N_1=7, N_2=9, NS), or bandwidth (U=45, N_1=9, N_2=11, NS). However, the pupal primary signal was several times louder than the larval grunt (M. Travassos, personal observation).

CONTEXT OF LARVAL SOUND PRODUCTION

Methods

We placed larvae on individual *Acacia* that had been cleared of ants and juvenile *J. evagoras*. Because calls can only be detected within a few centimeters of a caterpillar, we applied a band of Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan), a molasses-like substance, to the base of the host plant to limit the caterpillar's movements. We taped an accelerometer to a plant branch near the caterpillar.

After an acclimatization period of at least 30 min, we recorded calls in a 5-min control period through one channel of the tape recorder and spoken observations of the caterpillar's behaviour, coded as either stationary, walking (no feeding-related behaviour) or foraging (which included feeding), on the second channel. In addition, we also noted larval TO eversions in most time periods.

We used a wooden dowel to connect the host plant to an *I. anceps* colony. We again recorded the caterpillar's calls and behaviour in the 5 min following a worker ant's first contact with the caterpillar. Thirty minutes after an ant's discovery of the larva, we made a second 5-min recording.

We tested 11 larvae. Each caterpillar acted as its own control in comparisons of call production under different conditions.

Results

The presence of ants influenced the rate of larval sound production for two of the three types of calls (Fig. 2). Larvae produced significantly more grunts when first discovered by ants (Wilcoxon test: T=55, N=10, P<0.01) and after 30 min of ant attendance (T=55, N=10, P<0.01)

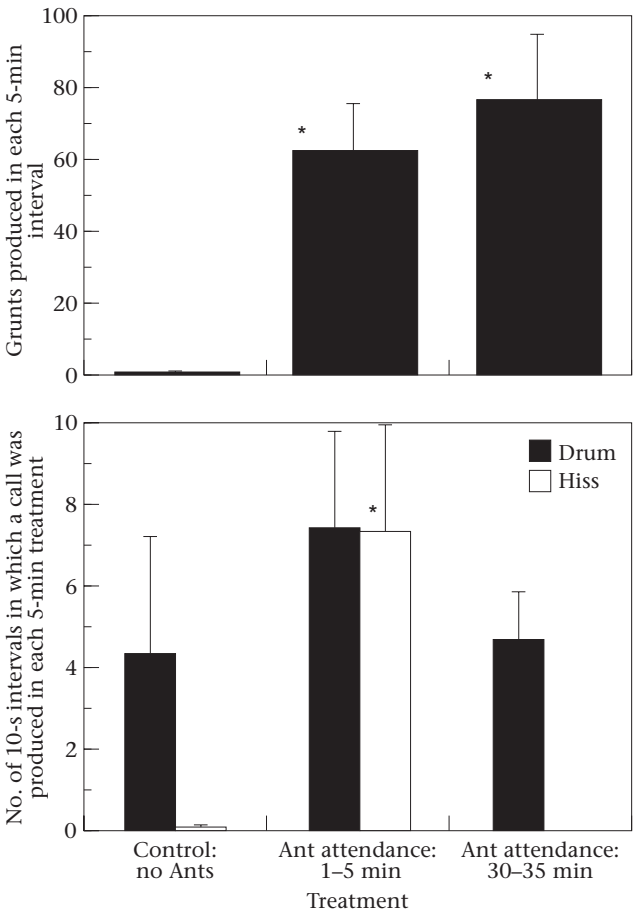

Figure 2. *Jalmenus evagoras* larval call production in the presence and absence of ants (*P<0.05, Wilcoxon signed-ranks test).

than in the control trial. Grunt call production did not differ between the two ant attendance intervals (T=30.50, N=9, NS). A caterpillar produced the hiss call when *I. anceps* workers first discovered it. Hiss call production was only detected once during the 5-min control period, but increased significantly once ants contacted the larva (T=34.50, N=10, P<0.05). However, after 30 min of ant contact, the hiss call was not produced. In contrast to the grunt and hiss call, the drum call was not produced significantly more when a caterpillar was attended by ants (χ^2_2=4.77, N=9, NS). However, in the first 5 min of ant attendance, there was a positive correlation between the amount of time spent foraging and the number of drum calls produced (Fig. 3). The more time a larva spent foraging when first discovered by ants, the more likely it was to produce a high number of drum calls. Correspondingly, during this time interval, there was a negative correlation between the amount of time a caterpillar was stationary and the number of drum calls produced (r_6= − 0.729, P<0.05). However, no such association existed between walking and drum call production (r_6=0.224, NS). In the second ant interval, there was no correlation between time spent foraging and drum calls produced (r_6= − 0.089, NS). There were no significant correlations between activity and the production of either hiss or grunt calls for any time interval.

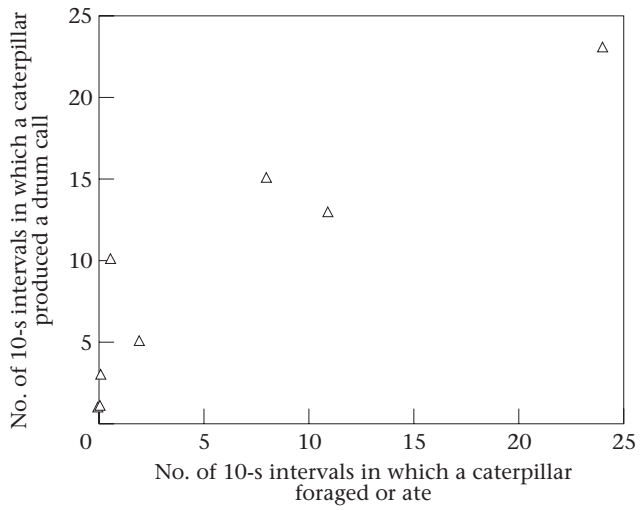

Figure 3. *Jalmenus evagoras* drum call production versus time spent foraging (10-s intervals), 5 min following discovery by attendant ants (r_6=0.908, P<0.001).

For seven out of nine caterpillars examined, tentacle eversions were strongly positively correlated with the production of grunts when ants were present (r_5=0.631 ± 0.044, P<0.01). Tentacle eversions were recorded for only two control periods, and in one of these, there was a significant correlation between tentacle eversions and grunts (r_5=0.695, P<0.0001). Tentacle eversions did not correlate with larval drumming or hissing in any context.

CONTEXT OF PUPAL SOUND PRODUCTION

Methods

Immediately before pupation, a final-instar larva was placed on a wooden stick (14.0 × 0.5 × 0.1 cm) held upright in a pool of water by a rubber stopper. The larva then pupated at the top of the stick.

In each trial, a rubber stopper held the stick upright on top of an inverted plastic cone that acted as a platform, suspending the pupa above the ant colony to which it was connected. An accelerometer was taped to the base of the pupation stick, just above the rubber stopper.

Before each trial, we recorded the length and width of each pupa and the weight of the combined pupa and stick. We weighed and sexed adults upon eclosion. In addition, we weighed the pupation stick after the cast pupal cuticle had been removed to estimate the weight of the pupa at the time of the trial. We also recorded pupation time (in days) to calculate the relative age of the pupa at the time of the trial (age in days at time of trial/total days in pupal period).

During some recordings, pulse trains of secondary signals accompanied primary signal production. Secondary signals were counted only when produced independently of primary signal production.

Resting call production

After an acclimatization period of at least 15 min, we recorded pupal activity for 5 min, during which the pupa was left undisturbed. This treatment acted as the baseline 'control'.

Defensive call production

We subjected each pupa to the following physical stimuli: we pinched the pupa with a pair of forceps to stimulate a predator attack (cf. Leimar & Axén 1993); we tapped the set-up rapidly at three locations (ca. 4 cm below the pupa, on the side of the stick directly opposite the pupa and directly on the pupal surface); we swept a paintbrush across the pupation stick 4 cm below the pupa; and brushed the pupal surface to simulate the antennae of ants or the hairs. We measured any calls produced after each stimulus.

Call production in the presence of a conspecific larva

We recorded calls in the 5 min after placing a fifth-instar caterpillar at the base of the pupation stick, and we noted movements of the caterpillar.

Call production in the presence of attendant ants

At the start of the trial, we connected the stick to an *I. anceps* colony using a wooden dowel. We recorded calls for 5 min following the discovery of the pupa by worker ants. To reach the pupa, each ant had to travel the length of the dowel bridge and then climb up the pupation stick. We counted the number of ants attending each pupa (the number of ants in contact with it) and the flow rate (the number of ants travelling up the dowel past a specific point in a 10-s period) once a minute. Thirty minutes after discovery of the pupa, we recorded calls for a second 5-min period.

We examined 19 pupae, but only the 16 pupae that eclosed successfully were included in the analysis. Each pupa served as its own control.

Statistical analyses

Calls produced in a stated time period are reported as means ± SE. Because levels of each call within each time interval were not normally distributed, we used nonparametric statistical tests to analyse the data. We made comparisons between pairs of treatments with Wilcoxon signed-ranks tests, and comparisons of more than two treatments with the Friedman's test (Sokal & Rohlf 1969). Ties were taken into consideration in examining significance. We used the Fisher's r to Z test (Abacus Concepts 1992) to assess significance in correlations and we used a more conservative level of significance (α=0.01) when analysing the variables affecting primary signal production.

Results

Resting call production

Each pupa produced 2.1 ± 0.7 primary signals and 2.9 ± 1.6 secondary signals in the 5-min period. Both

Table 2. Pupal sound production following the introduction of different stimuli (Wilcoxon signed-ranks test)

	Defensive stimuli						
Pupal call	Direct forceps pinch	Tap below	Tap behind	Tap directly on	Brush below	Brush directly on	Conspecific larva present
Primary signal							
N	9	13	14	15	13	13	15
P	<0.01	NS	NS	NS	<0.01	<0.01	<0.01
Secondary signal							
N	9	13	14	15	13	13	15
P	NS	NS	NS	NS	<0.05	<0.05	<0.05

kinds of signals were produced in single pulses and in trains.

Defensive call production

Calls produced in the 10 s following the application of certain stimuli were greater than the mean number of baseline calls produced over the equivalent time interval for each pupa (Table 2). For both paintbrush stimuli, production of primary and secondary signals was significantly greater than resting levels. When brushed on its cuticle, a pupa produced significantly more primary signals than it did when the brush was applied to the wooden stick (Wilcoxon test: T=78, N=13, P<0.01). However, secondary signal call production did not differ between these two treatments (T=16.50, N=12, NS).

Pinching the pupa with forceps ('predator attack'), produced a significantly greater primary signal than during the control treatment (T=45, N=9, P<0.01), but secondary signal production did not differ from the control (T=12, N=9, NS). The number of primary signals produced by each pupa when 'attacked' did not differ from the number it produced when the pupation stick (T=22.50, N=9, NS) or the pupa's cuticle (T=36.50, N=9, NS) was brushed.

Call production following rapid taps did not differ significantly from that produced at resting levels.

Call production in the presence of a conspecific larva

Primary signals were produced throughout the 5 min after introduction of a conspecific larva (Table 2). Pupal primary signals of *J. evagoras* have greater amplitude than larval grunts and so were clearly distinguishable. Significantly more primary and secondary signals were produced in the presence of a larva than were produced in the control treatment (Wilcoxon test: primary signals: $Z=-3.23$, N=15, P<0.01; secondary signals: $Z=-2.09$, N=15, P<0.05). Call production/min did not change significantly over the 5-min treatment for the primary (χ^2_4=2.23, N=12, NS) or secondary signal (χ^2_4=3.03, N=12, NS). However, immediately after the introduction of the pupation stick, the caterpillar began to move, producing vibrations that were audible through the accelerometer. In all cases, the caterpillar climbed to the top of the pupation stick. Pupal calls were produced most frequently when the caterpillar was moving. The caterpillar moved most at the beginning of the 5-min interval and less so as time progressed. Reflecting this, the mean number of pupal primary signals/min decreased over time.

Call production in the presence of attendant ants

While secondary signal production did not differ significantly from that of resting levels in the same time interval (Wilcoxon test: $Z=-0.56$, N=15, NS), primary signal production in the first 5 min of ant attendance was greater than that in the control treatment ($Z=-3.41$, N=15, P<0.001). Primary signal production decreased from 1 to 5 min (χ^2_4=25.54, N=13, P<0.0001); however, this drop was only significant between 4 and 5 min (T=80, N=13, P=<0.05). Thirty minutes after ants had discovered the pupa, primary call production/min had decreased three-fold, but was still significantly greater than call production by the pupae over a 5-min control interval ($Z=-3.41$, N=15, P<0.001). Although secondary signal production increased during this interval, it did not differ significantly from that produced during the control period ($Z=-1.61$, N=15, NS).

In the 5 min following discovery of the pupa, ant attendance doubled (Fig. 4), and had doubled again after 30 min. Between 30 and 35 min, ant attendance levels remained constant (χ^2_4=6.91, N=13, NS). The ant flow rate fluctuated in the first 5-min interval but stabilized after 30 min at just over two ants per 10-s interval (χ^2_4=4.29, N=10, NS).

Ant flow increased as primary signal production increased (Fig. 5). These two variables were positively correlated (r_{128}=0.341, P<0.0001).

Categorical variables affecting primary signal production

Colony. There were no differences in signal production for Ebor (N=9 pupae) and Canberra (N=7 pupae) populations for any treatment.

Sex. Although female pupae (N=7) weighed more than male pupae (N=9), primary signal production did not correlate with pupal weight for any treatment. As a result, we did not include variance in weight as a factor when examining sex differences in primary signal output. No significant sex differences were found in call production for any treatment.

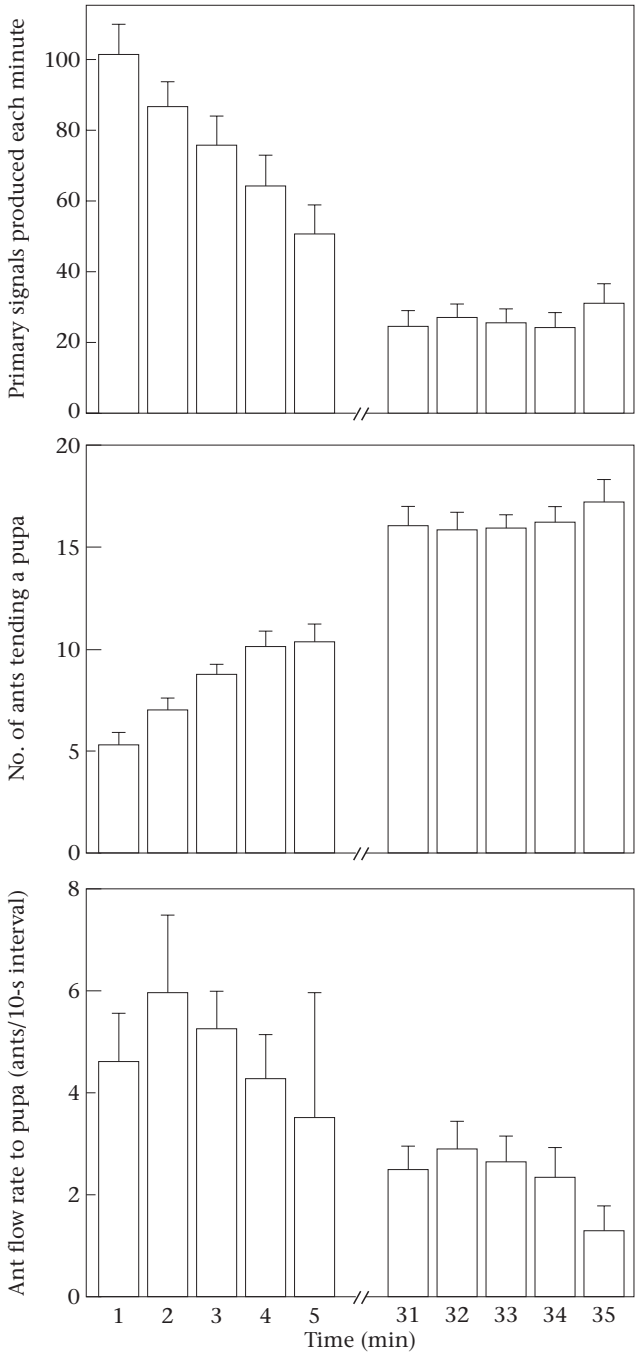

Figure 4. Mean primary signal production, ant attendance and ant flow following the discovery of a pupa by attendant ants (Wilcoxon signed-ranks test: $P<0.001$).

Noncategorical variables affecting call production

Neither adult weight, pupal weight nor pupal size correlated with results from any treatment. However, in the presence of ants, relative pupal age (as a fraction of total days since pupation) was strongly correlated with secondary signals produced/min in both the first ($r_{14}=-0.688$, $P<0.01$) and the second ($r_{14}=-0.648$, $P<0.01$) time interval. When attended by ants, older pupae thus produced fewer secondary signals over time than their younger counterparts.

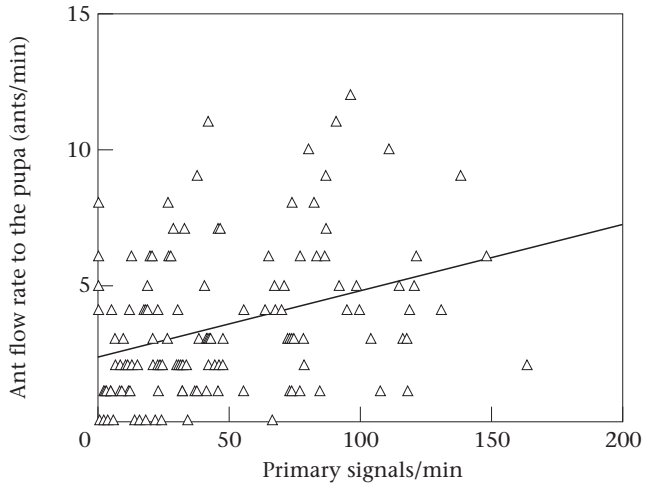

Figure 5. Ant flow rate versus primary signal production by pupae of *J. evagoras*. As a pupa increased its primary signal production, the recruitment rate of ants to the pupa also increased ($r_{128}=0.341$, $P<0.0001$).

THE FUNCTION OF PUPAL CALL PRODUCTION

Methods

Subjects

We separated pupae by area of origin (Ebor versus Mount Nebo, etc.), grouped them according to date of pupation and paired them off by size and weight. We occluded the stridulatory organ of a randomly assigned pupa in each pair with NutraNail® 60 Second One Coat Clear Gloss nail polish (CCA Industries, Inc., East Rutherford, New Jersey), which we applied between the fifth and sixth abdominal segments to prevent the teeth from rubbing against the opposing file. We confirmed with an accelerometer that the nail polish prevented the pupae from producing any calls, and a dissecting microscope examination revealed that it prevented any visible contractions of the pupal integument. As a control, nail polish was placed on the posterior edge of the fifth abdominal segment of the other pupa in the pair, close to, but not on, the stridulatory organ. There was no significant difference between the pupal weight of the control and experimentally manipulated pupae (Wilcoxon test: $Z=-1.05$, $N=39$, NS).

Attractiveness of pupal sound to larvae

We constructed a Y-shaped set-up for each pupal pair using the previously described pupation sticks of each pair as arms, wedged into opposing notches carved into the top of a 1.0-cm wooden dowel, (1 cm diameter, 23 cm long; Fig. 6a). A rubber stopper acted as a base, keeping the dowel upright. At the start of each trial, we placed a cylinder of black paper around the set-up to eliminate any external stimuli. We tested two groups of caterpillars: fifth-instar larvae and 1-day-old first instars. In each 60-min trial, we placed a caterpillar on the dowel 3 cm below the Y-junction, with its head oriented upwards. We placed a fluorescent lamp directly above the set-up to induce positive phototaxis. We scored a larva's preference

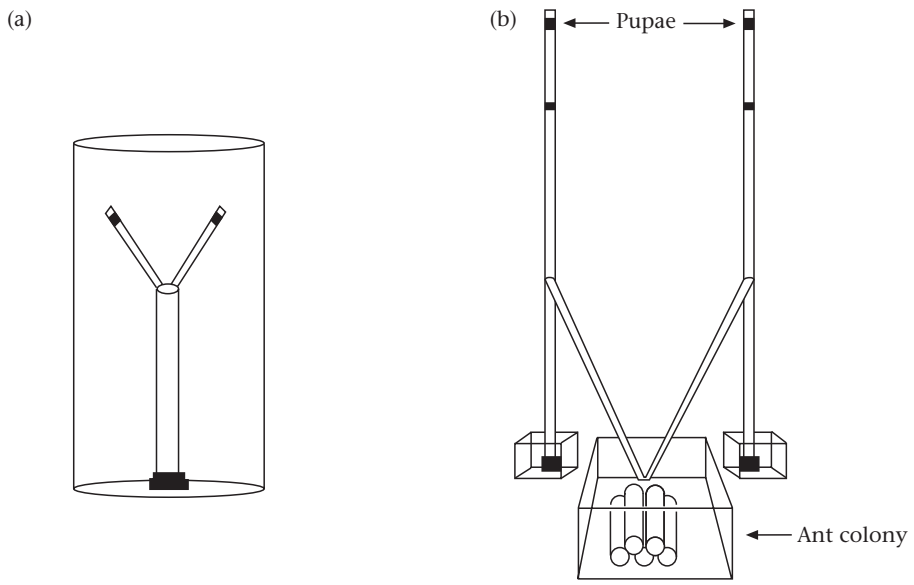

Figure 6. Set-ups for (a) larval choice and (b) ant preference trials.

for one of the two pupae if, after coming in contact with both pupae, it touched one of the pupae following its return to the dowel platform. If the caterpillar remained with one pupa for the duration of the trial either without coming in contact with the other pupa or without returning to the dowel platform after visiting both pupae, that pupa was counted as its choice. We performed 35 trials with first instars and 25 trials with fifth instars.

Attractiveness of pupal sound to attendant ants

To compensate for differences in pupa location on pupation sticks, we trimmed the two sticks to have identical lengths both above and below each pupa. We then weighed the sticks and pupae. We glued each stick to a dowel (0.3 cm diameter, 60 cm long), to which we had glued a second dowel (0.3 cm diameter, 37 cm long) 27 cm from the base at an angle, forming a supporting leg to the base.

At the start of each trial, we introduced both dowel set-ups of a pupal pair into a queenright *I. anceps* colony. We positioned the bridges next to each other in the ant colony and placed each base in a small pool of water to prevent ants from leaving the set-up (Fig. 6b). The ants thus had two bridges to choose from: one leading to a calling pupa, the other to its muted partner. To reach a pupa, ants had to travel the length of the bridge and then climb up the dowel to the pupation stick. We counted the number of ants attending each pupa, measured as the number of ants in contact with it, after 20, 40, 60 and 100 min. We also determined the flow rate, measured as the number of ants travelling up the dowel past a specific point in a 10-s period at each of these time intervals. We tested 10 different pairs of pupae on each of five *I. anceps* colonies. After each trial, we used new dowels and bridges to remove possible traces of ant trail pheromones. We conducted 49 trials.

Upon metamorphosis into an adult, we recorded the wing length, weight and sex of each butterfly. After removing the pupal skins, we weighed each stick again, so that we could estimate the pupal weight at the time of the experiment.

Statistical analyses

We analysed larval preference tests with a one-sample sign test. For the ant preference tests, we examined colony effects at each time interval with a Kruskal–Wallis test to determine whether there was a significant difference in ant attendance levels between the five colonies. Because ant attendance levels were not normally distributed at each time interval, we used nonparametric tests. We compared differences in discovery time and ant attendance levels at each census time with the Wilcoxon signed-ranks test and made matched comparisons of more than two groups with the Friedman's test (Sokal & Rohlf 1969). We used the Mann–Whitney *U* test for those nonmatched comparisons between two groups and the Kruskal–Wallis test (Sokal & Rohlf 1969) for those between more than two groups. We used the Fisher's *r* to *Z* test (Abacus Concepts 1992) to assess significance in correlations. Significance values were adjusted for ties. We used a more conservative level of significance (α=0.01) to determine the factors influencing ant attendance levels. Because the residuals from the least squares regression of pupal weight on ant attendance were normally distributed, we analysed comparisons between males and females with a one-factor analysis of variance. All quantities are reported as means ± SE.

Results

Attractiveness of pupal sound to larvae

First-instar larvae chose the stridulating pupa 22 times in 35 trials, a result that did not differ significantly from that of random choice (*N*=35, NS). Similarly, fifth-instar caterpillars did not display a preference for the calling pupa, choosing it 12 times in 25 trials (*N*=25, NS).

Table 3. Kruskal–Wallis test for intercolony variation in ant attendance levels

Time (min)	Control pupae		Painted pupae	
	H	*P*	*H*	*P*
20	2.644	0.62	8.480	0.08
40	5.147	0.27	7.838	0.10
60	8.134	0.09	7.146	0.13
100	8.527	0.07	4.839	0.30

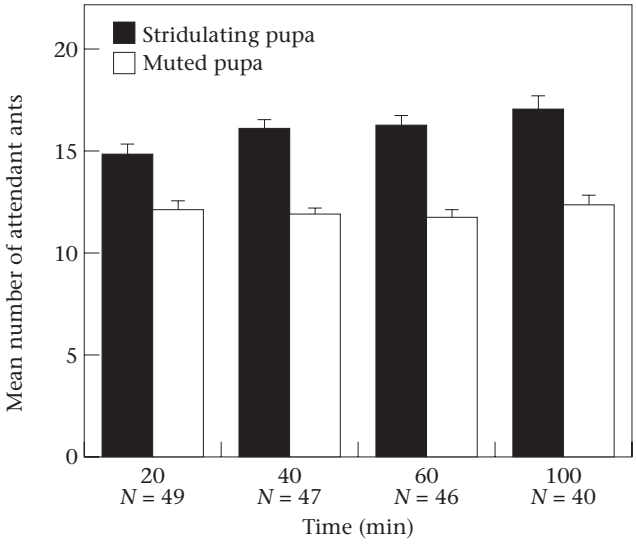

Figure 7. Stridulating pupae were attended by significantly more ants than were muted pupae at all time intervals (Wilcoxon signed-ranks test: $P<0.0001$).

Attractiveness of pupal sound to attendant ants

Discovery time. The difference in elapsed time until discovery did not differ between stridulating and muted pupae (Wilcoxon test: $Z=-0.95$, $N=49$, NS). Stridulating pupae were discovered by *I. anceps* workers after 50.9 ± 5.3 s, while muted pupae were discovered after 53.9 ± 5.7 s.

Ant attendance. As indicated in Table 3, ant attendance levels did not differ between ant colonies for either control or painted pupae at any of the four time intervals, thus making it feasible to pool the results from the five colonies. Stridulating pupae maintained a higher level of ant attendance than did their muted counterparts at all four time intervals (Fig. 7). For stridulating pupae, ant attendance levels increased between 20 and 40 min (Wilcoxon test: $Z=-3.09$, $N=47$, $P<0.01$) but then leveled off (40–60 min: $Z=-0.53$, $N=46$, NS; 60–100 min: $Z=-1.64$, $N=40$, NS). Ant attendance levels for muted pupae did not vary over time ($\chi^2_3=3.97$, $N=40$, NS).

Ant flow rates. While the mean flow rate of ants visiting control pupae was higher than that of muted pupae at all four time intervals (Fig. 8), it approached significance at 20 min (Wilcoxon test: $Z=-3.09$, $N=48$, $P=0.06$) and was significantly different at 100 min ($Z=-2.66$, $N=38$, $P<0.01$). The mean flow rate for both pupal groups decreased over time, and this decline was more pronounced for flow rates to muted pupae. Changes in ant flow rates over time were not significant for either control ant flow rates ($\chi^2_3=0.46$, $N=35$, NS) or muted pupae ant flow rates ($\chi^2_3=3.91$, $N=35$, NS).

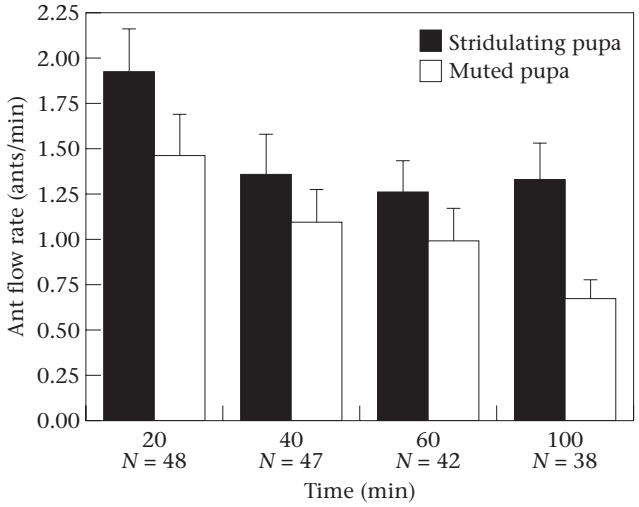

Figure 8. Although the ant flow rate of the stridulating pupa was higher than that of the muted pupa at all four census times, this difference was only significant at 100 min (Wilcoxon signed-ranks test: $P<0.01$).

Factors influencing ant attendance levels

Because treatment significantly affected pupal ant attendance levels, pupae were separated into control and muted groups to determine other factors influencing ant attendance levels.

Pupal weight. There was a positive (linear) correlation between pupal weight and ant attendance levels in both control and muted pupae at all four census times (Fig. 9).

Because pupal weight and ant attendance levels were strongly correlated, we calculated a least squares regression of pupal weight on ant attendance and examined the effects of other factors in relation to the residuals thus obtained.

Sex. Although female pupae are heavier than males (Pierce & Elgar 1985), the effects of pupal weight were removed by considering the residuals from a least squares regression of pupal weight on ant attendance levels. Male and female pupae in both the control and muted treatment did not differ significantly in the numbers of attendant ants attracted at any time interval.

Age. There was no significant correlation between relative pupal age and attendant ant levels at any time interval.

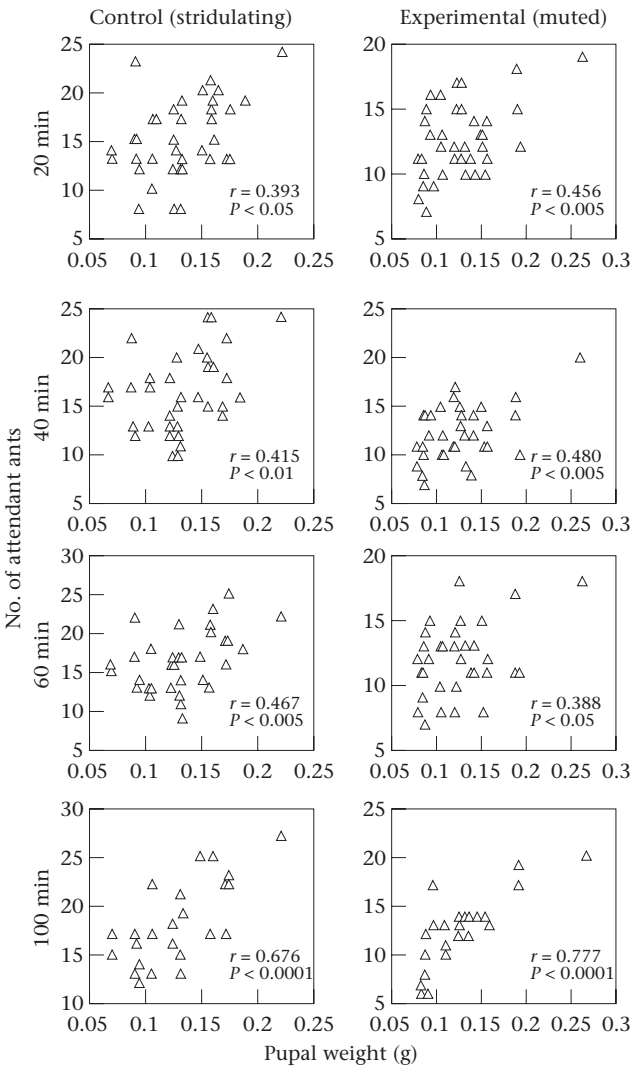

Figure 9. The correlation between the numbers of attendant ants and pupal weight for both control and painted pupae at all time intervals censused.

Adult weight and wing length. Pupal weight was correlated with adult weight (r_{36}=0.873, P<0.0001) and wing length (r_{36}=0.789, P<0.0001). However, in terms of least squares regression residuals, there was no correlation between adult weight and attendant ant levels or wing length and attendant ant levels.

DISCUSSION

Pupae of *J. evagoras* produce a single call type that contains both primary and secondary signal elements; larvae produce three distinct calls. Pupal sound production occurs in several different contexts: at rest, when disturbed, with the introduction of a conspecific larva, and in the presence of attendant ants of *I. anceps*. These calls are important in attracting ants to *J. evagoras* and maintaining a high level of ant attendance over time. Calling was correlated with the rate at which ants visited pupae, showing that calling pupae not only maintain a higher ant attendance level, but also induce a greater recruitment and turnover rate of workers.

Two of the three vibrational signals produced by *J. evagoras* larvae have distinct contexts in ant interactions: grunts, which share several acoustic properties with pupal primary signals and are produced during ant attendance; and hisses, which are produced in the first 5 min after discovery by a worker ant. Drumming, however, is equally likely to be produced by both tended and untended larvae. Interestingly, larvae that were actively foraging when first discovered by ants were more likely to produce drum calls than their counterparts that were resting or travelling from one location to another. While we have not demonstrated that either *I. anceps* or *J. evagoras* juveniles can discriminate between these three signals, given their different contexts, it is likely that each one has a distinct function.

Taken together, these results indicate that vibrational communication plays a significant role in the interaction between *J. evagoras* and its associated ants, *I. anceps*. While previous studies have suggested that lycaenid pupal sound production is largely defensive (Hoegh-Guldberg 1972; Downey & Allyn 1978), our experiments show that by producing and varying primary signal production, a pupa may adjust the size of its ant guard, a result similar to that shown by DeVries (1990) for a riodinid caterpillar. *Jalmenus evagoras* pupae are unusual in having heavily ant-attended pupae, and so our results may not be applicable to all lycaenid taxa. It is also important to note that sound production in the presence of ants cannot be explained as merely a response to a disturbance. If this were true, the number of primary signals would increase with increasing numbers of attending ants. Instead, primary signal production decreased as ant attendance initially increased, and when ant attendance numbers stabilized, primary signal production did also (Fig. 4). The disturbance hypothesis also suggests that pupal calling is merely defensive and holds no attractive value for ants; we have shown that stridulating pupae attract and maintain associations with more ants than muted pupae do.

Like pupae, larvae increase their production of vibrational signals in the presence of *I. anceps*, suggesting that larval sound production plays a similar role in ant interactions as pupal calls. In addition, larval grunts and pupal primary signals share the same mean dominant frequency, pulse rate, bandwidth and pulse length. In the presence of ants, larvae do not produce grunts independent of tentacle organ eversions; instead, a caterpillar is likely to produce grunts and TO eversions in tandem, suggesting that substrate-borne vibrations may work in concert with the TOs to modify ant behaviour.

Axén et al. (1996) emphasized that participants in mutualistic relationships may attract partners with the use of signals, to induce them to initiate interactions. These signals may also be important in regulating interactions. We have shown that pupal primary signals play such a role in pupa–ant interactions. The highest level of call production in any pupal treatment occurred in the first minute following an ant's discovery of a pupa. To ants, pupal calls may signal a wise investment: a healthy

pupa that will provide valuable secretions. Considering the high levels of pupal sound production in the presence of ants, there may be a significant metabolic cost associated with stridulation; 30 min after ants discovered a pupa, heavier pupae produced a greater number of secondary signals than their lighter counterparts. In addition, three pupae that did not survive to eclosion produced fewer primary signals when tended by ants than did their healthier counterparts, suggesting that calling may be energetically costly. High sound production may thus be an honest signal to ants of lycaenid quality, indicating a heavy, healthy pupa. At the same time, the ability to affect attendant ant numbers by varying call production would be an important adaptation for lycaenids, because although ants are critical to pupal survival, maintaining an ant guard is costly. Pierce et al. (1987) found that pupae tended by ants lost 25% more weight than untended pupae and took longer to eclose. Primary signal production may allow a pupa to adjust its attendant ant numbers so that it receives adequate protection from predators and parasites, but also limits costly energy expenditures in the form of secretions. When a pupa increased primary signal production levels when tended by *I. anceps*, there was a greater flow rate of attendant ants to the pupa. This may be because an increase in the rate of primary signals alarms *I. anceps* workers or because the production of such signals is directly correlated with pupal secretion rates (i.e. when a pupa increases call production, it might also increase secretions to attendant ants, resulting in higher recruitment levels of workers to the pupa).

In demonstrating the significance of sound production in a lycaenid–ant association, we provide support for DeVries's (1991a) hypothesis that vibrational communication is common to all ant trophobionts. Previous work has shown that myrmecophiles have broken the tactile and chemical communication codes used by ants. The staphylinid beetle *Atemeles pubicollis*, for example, uses tactile stimulation to solicit food from ants, inducing regurgitation by stroking the ant in a specialized manner (Hölldobler 1971), while the beetle *Myrmecaphodius excavaticollis* acquires the species-specific cuticular hydrocarbons of its host ants, enabling it to live in a colony and use its food resources (Vander Meer & Wojcik 1982). The extent to which such myrmecophiles use vibrational communication to interact with ants has not been fully explored. The results presented here, together with DeVries's (1990) finding that substrate-borne vibrations are important in riodinid–ant interactions, suggest that it might be useful to investigate the role of vibrational communication in other ant associations as well.

Substrate-borne vibrations have long been known to stimulate ant interest (Fielde & Parker 1904), and ants use stridulation, drumming and rapid vibrations of the head and thorax to communicate. Baroni-Urbani et al. (1988) demonstrated that stridulation in *Messor capitatus* is important in recruiting workers to a food source quickly and that the calls are primarily perceived as substrate vibrations by nestmates. In the leaf-cutting ant *Atta cephalotes*, a worker stridulates when cutting an attractive leaf; the vibrations attract other workers (Roces et al. 1993). Stridulatory organs have been found in several subfamilies of ants, including Pseudomyrmecinae, Myrmicinae and Ponerinae (Markl 1973). Markl (1973) suggested that such organs are found primarily in ants with terrestrial nests. An examination of the Dolichoderinae, the subfamily to which *Iridomyrmex* belongs, did not reveal the presence of such a sound-producing organ (Markl 1973). Nevertheless, vibrations play a role in communication in some dolichoderine ants. Certain arboreal nest-dwellers, including members of the Dolichoderinae, drum body parts such as the gaster against the substrate in certain contexts, producing vibrations that trigger a 'stop' or 'run' reaction in workers (Fuchs 1976; Hölldobler & Wilson 1990). Several primitive species in the poneroid complex of ants also communicate via vibrations. Although they lack a stridulatory organ, they produce vibrational displays of the head and thorax that are important in alarm communication (Hölldobler 1977; Traniello 1982).

While none of these forms of vibrational communication is known in *Iridomyrmex*, we have observed that *I. anceps* workers are extremely sensitive to substrate vibrations. When disturbed, *J. evagoras* pupae produce pulses that travel the length of a pupation stick (personal observation). These substrate-borne signals may be important attractants to worker ants, but there may also be visual and tactile components to this enticement. Observations with a dissecting microscope revealed that the pupal integument of *J. evagoras* trembles with each call. When larvae grunt, body segments contract and sometimes thrash. Ants can perceive rapid movement (Voss 1967) and run towards moving prey, suggesting that rapid movements may stimulate them (Malicky 1970; Wilson 1971). In the genus *Amblyopone* (Formicidae: Ponerinae), nestmates become alarmed when touching workers displaying 'vigorous jittering behaviour, consisting of rapid vertical movements of the head and thorax' (Traniello 1982, page 73). Pulses transmitted to workers of *I. anceps* in contact with the pupa or larva may be a way of maintaining their interest. However, airborne components of sounds from *J. evagoras* juveniles probably do not play a role in interactions with *I. anceps*, because ants are nearly deaf to airborne vibrations (Hölldobler & Wilson 1990).

Weight and age influence sound production in pupae. Heavier pupae attracted and maintained a larger retinue of ants than their lighter counterparts. Younger pupae produced a greater number of secondary signals than their older counterparts in the first 35 min of ant attendance.

Although the acoustic characteristics of vibrational signals reported here for *J. evagoras* differ from those reported by DeVries (1991a), this may be the result of the use of different substrates when recording. The larval call reported by DeVries (1991a) has a similar pulse rate to the hiss call, which is produced when larvae are disturbed; however, the mean dominant frequency reported here for this call is 471.7 Hz, while DeVries (1991a) observed a frequency of 1700 Hz. The frequency of the pupal primary signal we report is also different. However, DeVries (1991b) tested lycaenid caterpillars and pupae on a taut membrane sandwiched between two petri dishes,

stimulating the subject with entomological forceps. In this experiment, larvae were tested on their host plants, while pupae were tested on wooden sticks. As Michelsen et al. (1982) demonstrated, the parameters of insect vibrational songs may be affected by the mechanical properties of the substrate on which they rest.

Our results also differ from previous work because of the way we stimulated lycaenid juveniles to stridulate. Previous studies have induced calls by disturbing caterpillars (DeVries 1991b; Fiedler et al. 1994; Schurian 1995); by this method, one larval call was found for *J. evagoras* (DeVries 1991a). In our study, caterpillars produced three distinct calls when tended by ants, suggesting that lycaenid larvae may have a larger repertoire of calls than is currently known.

The role of vibrational signals in defence and the formation of juvenile aggregations appears to be indirect. Pupae called in the presence of fifth instar *J. evagoras* larvae, although this happened mostly when the caterpillar was moving and thereby producing vibrations. Whether calling is specific to conspecific larvae or is a general response to any kind of vibration was not tested. Larval choice experiments did not demonstrate a significant larval preference for pupal stridulation. However, work by Mathews (1993) demonstrated that larvae follow ant trails, suggesting that this may be one mechanism juveniles use to form aggregations. If pupal sounds are important in attracting and maintaining ant associations, such sounds may therefore play an indirect role in the creation of aggregations. In addition, pupae selectively produced calls in response to different physical stimuli, and this may serve to regulate the size of the ant guard under natural conditions. Thus, stridulation may function as a defensive mechanism as well as an ant lure.

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