OFFSPRING-PARENT COMMUNICATION IN A SUBSOCIAL TREEHOPPER (HEMIPTERA: MEMBRACIDAE: UMBONIA CRASSICORNIS)

by

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Summary

Parental care of post-hatching offspring is widespread in insects, but the role of communication in parent-offspring interactions remains largely unknown. I have found that, in the subsocial treehopper *Umbonia crassicornis*, aggregated nymphal offspring produce substrate-borne, vibrational signals in synchronized bursts that elicit the mother's antipredator behavior. In this study I describe the signals used by nymphs and explore their role in mother-offspring interactions and within-brood communication. Nymphs were stimulated to signal in the laboratory in response to light contact, simulating the approach of a predator. Signals of nymphs at the site of disturbance triggered a rapid wave of signaling by many individuals within the aggregation. This coordinated signaling was associated with the mother's defensive behavior. Signaling was limited to the vibrational channel: when transmission of vibrations was blocked between signaling nymphs and the mother, the mothers' response was abolished. Nymphs signaled not only in response to contact, but also in response to playback of signals from their siblings. Nymphs in otherwise undisturbed aggregations signaled only in response to signals coordinated into synchronized, group displays, and not to signals in random temporal patterns. However, nymphal signaling thresholds were lowered after a recent experience of simulated predation. After a period in which nymphs were stimulated to signal (by light contact simulating a predator's approach), playback of one

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individual signal could trigger a coordinated burst within the aggregation. It remains unknown if coordination among siblings to produce synchronized, group signals is completely cooperative, or if siblings compete for the mother's proximity. But it is clear that a complex system of communication among siblings, and between siblings and their parent, is an important feature of maternal care in these subsocial insects.

Introduction

Group living creates the potential for a wide range of social interactions. The existence of communication systems makes those interactions possible, and communication is a general feature of the biology of group-living animals (Wilson, 1975; Sherman, 1977; Klump & Schalter, 1984; Cheney & Seyfarth, 1990; Fitzgerald, 1995). The intimate relationship between communication and sociality is well illustrated by eusocial taxa such as ants, naked mole rats, and honeybees, in which complex systems of communication among colony members allow cooperative, colony-level responses to changes in the environment (Wilson, 1971; Holldobler & Wilson, 1990; Seeley, 1995; Judd & Sherman, 1996) as well as competitive interactions among individuals (West-Eberhard, 1983, 1984; Keller & Nonacs, 1993). Communication is also important in family groups in which parents provide resources to offspring. Because offspring can use signals to solicit these resources, communication is of interest for its potential role in the expression and resolution of parent-offspring conflict and sibling rivalry (Trivers, 1974; Stamps *et al.*, 1985; Lazarus & Inglis, 1986; Godfray, 1995).

Although communication in parent-offspring groups has been extensively studied in birds and mammals (Clutton-Brock, 1991), its role is largely unknown in the analogous social groups in insects. Parental care of post-hatching nymphs or larvae (subsocial behavior) is widespread in insects (Tallamy & Schaefer, 1997). Given the diversity of ecology and life history among insects with parental care, including differences in clutch size, within-clutch relatedness, number of broods per female, and the nature of the resources provided by parents (Wilson, 1971; Eickwort, 1981; Tallamy & Wood, 1986; Tallamy, 1994; Tallamy & Schaefer 1997), we might also expect a diversity of offspring-parent communication systems. Observations on a few groups suggest this may be the case. In burying beetles, tactile or vibrational signals are likely involved in parent-offspring

interactions (Lengerken, 1954; Huerta *et al.*, 1993), while in group-living passalid beetles, acoustic signals have been documented but their function is unknown (Buchler *et al.*, 1981; Schuster & Schuster, 1997). In an aepophilid bug, presumed tactile signals from the mother appear to influence nymphal dispersal (Keys, 1914). In several subsocial Hemiptera, parents have a chemically-mediated alarm response to injury of their offspring (Nault & Phelan, 1984), although it is not always clear that this involves specially-evolved signals.

In this study I examine the role of parent-offspring communication within family groups of a subsocial treehopper (Hemiptera: Membracidae; *Umbonia crassicornis*). The treehoppers are a diverse group of sap-feeding insects in which many species have some form of parental care (Wood, 1979, 1984). Treehoppers recently have been shown to communicate by means of substrate-borne vibrations (Hunt, 1993, 1994; Cocroft, 1996), a signaling modality characteristic of many small insects (Claridge, 1985; Gogala, 1985; Henry, 1994).

Female *U. crassicornis* are semelparous, depositing a single clutch of approximately 100 eggs into a host plant stem (Wood, 1983). Females typically spend the rest of their lives caring for that clutch of offspring, remaining with them until they mature (Wood, 1975, 1983, 1985). Members of a brood are likely to be full siblings, because females usually mate singly (Wood, 1983) and apparently do not deposit eggs in the clutches of other females (as do females in some subsocial insects: Tallamy, 1985; Eberhard, 1986). However, relatedness within groups may sometimes be lower, as when females mate multiply (K. Masters, pers. comm.) or when two or more clutches on the same stem merge and are tended jointly (pers. obs.). After hatching, nymphs form a tight, stationary aggregation along the host plant stem. The female remains on the stem below the nymphs and provides two classes of resources. The first is nutritional: the female uses her ovipositor to make a series of punctures in the stem below the egg mass, through which the nymphs feed (Wood, 1974). The second is protection against predators.

Nymphal *Umbonia* are subject to intense predation in their exposed locations on plant stems. Their predators include vespid wasps (Dowell & Johnson, 1986; Cocroft, 1996), predatory Hemiptera, syrphid fly larvae, neuropteran larvae, and coccinellid beetles (Wood, 1976, 1983;

Masters, 1989; McKamey & Deitz, 1996; Cocroft, unpub. data). The mother's active defense is the nymphs' main protection when attacked by invertebrate predators. Females defend their offspring by approaching the predator, fanning their wings, and kicking with their hind legs (Brach, 1975; Wood, 1975, 1976, 1983; Dowell & Johnson, 1986; McKamey & Deitz, 1996). Disappearance of the female results in greatly increased predation on nymphs (Wood, 1975; Dowell & Johnson, 1986; Cocroft, 1998).

I have found that *U. crassicornis* nymphs communicate to obtain maternal defense against predators (Cocroft, 1996). When approached by a predator, nymphs at the site of disturbance produce substrate-borne vibrational signals. These signals trigger a response from neighbors, and signaling sweeps rapidly through the aggregation. As a result, the signals of individual nymphs combine to form a coordinated 'group' signal, which is longer and greater in amplitude than an individual signal. Group signals evoke the mother's defensive behavior. Importantly, individual signals are effective in eliciting maternal care only when they are coordinated into synchronous displays (Cocroft, 1996). This coordination may represent cooperation among siblings in soliciting maternal care. However, it is also possible that nymphs may be able to compete for the mother's proximity. Understanding how selection acts on the behavior of signaling offspring within aggregations will require a more detailed characterization of the context, and consequences, of nymphal signaling.

In this report I describe the signals of *U. crassicornis* nymphs and further explore their role in within-group interactions. First, I address the context in which nymphal group signals, and the mother's response, can be evoked experimentally. Second, because Wood (1976) showed that female *U. crassicornis* can respond to nymphal injury using chemical cues, I investigate whether antipredator signaling is limited to vibrations traveling along the host plant stem. Finally, I explore the conditions under which nymphs will signal in response to signals from their siblings. Is temporal coordination of signals important for communication within the brood, as it is for communication between the brood and the parent? And do nymphal signaling thresholds change as a result of experience, decreasing after a recent predator encounter?

Methods

Umbonia crassicornis occurs from northern South America through Mexico, with an introduced population in southern Florida, USA (McKamey & Deitz, 1996). I collected adults from *Albizia lebbeck* and *Calliandra haematocephala* (Leguminosae: Mimosoideae) near Sarasota, Florida, and established a greenhouse population on potted host plants (*A. lebbeck*, *A. julibrissin*, and *C. haematocephala*). The greenhouse temperature ranged from 20° to 30°C, and lighting was on a 12 h light/12 h dark schedule. I made field collections of treehoppers every three generations to reduce the potential of inbreeding.

I recorded vibrational signals of nymphal *U. crassicornis* by glueing an accelerometer (Knowles BU-1771, weight 0.28 g) to the host plant stem 1-2 cm from the aggregation. Hosts used by the insects in the greenhouse colony were robust, woody plants 1-1.5 m tall. Laser doppler vibrometry (Polytec OFV 3000 vibrometer controller, OFV 302 sensor head) recordings of *A. julibrissin* stems with and without the accelerometer and cable attached showed that mass loading by the accelerometer had a minimal impact on the transmission characteristics of the vibrational channel (average effect was < 1 dB across the relevant frequency range, about equal to the loading effect of 2-4 adult insects; R. Cocroft and R. Miles, unpub. data). Frequency response of the accelerometer was flat (± 3 dB) from 20-5500 Hz. Signals were amplified using a customized operational amplifier and recorded using either a Sony TC-D5M or WM-D6C cassette recorder. I made all recordings at $27 + 1$°C, and used only 3rd to 5th instar nymphs in the study.

I conducted vibrational playbacks using either (1) an electrodynamic shaker (Labworks ET 132-203), with signals coupled to the host plant using a bolt extending 3 mm from the armature mounting stud and lightly pressed against the stem; or (2) a magnet glued to the stem and driven with an electromagnet (Michelsen *et al*., 1982). Signals were played from a MacIntosh IIsi computer and routed to the shaker through a Radio Shack MPA-45 amplifier. I recorded playbacks on videotape using a Nikon VN-950 Hi-8 camcorder.

Undesirable frequency filtering of the playback signal can be caused at various stages of a vibrational playback system, including the transducer and the host plant stem. I dealt with this problem as follows (also see Cocroft, 1996). First, I recorded nymphal signals with an accelerometer attached to the stem within the aggregation. Second, prior to each playback, I estimated the filter characteristics of the system (computer output, shaker, host plant stem) by playing random noise (flat from 50-5500 Hz) through the shaker or magnet/electromagnet combination (positioned at the edge of the aggregation) and recording it with the accelerometer. I calculated the filtering effect of the system, then inverted this filter and used the result to create a digital filter that I applied to the noise signal. I reiterated this procedure (usually twice) until the played-back noise as recorded by the accelerometer also had a flat spectrum (to within 1 dB) from 50-5500 Hz. I then applied the set of filter coefficients sequentially to the experimental playback stimuli; this compensated for the filtering effects of the system to produce high-fidelity playback of nymphal signals. I adjusted playback signals to be within 5% of their peak amplitude when originally recorded, as monitored by the accelerometer.

I played the stimuli from a MacIntosh IIsi computer, using SoundEdit (Farallon, Inc.). I digitized signals with a MacRecorder digitizer (sampling rate 22 kHz) and analyzed them using Canary (Cornell Laboratory of Ornithology, Ithaca, NY, USA). I performed the digital filtering for frequency correction using MATLAB (The Math Works, Inc., Natick, MA, USA).

Experimental design

Experimentally evoking nymphal signals and maternal response

Observations in the field showed that nymphal group signaling was correlated with the presence of a predator (Cocroft, 1996, in press). I wished to determine whether I could cause nymphs to signal in the laboratory by experimentally simulating a predator's presence. I created an experimental disturbance by attaching fine bristles from a watercolor brush to the end of a rod mounted on a pen motor. The motor was positioned on a tripod and driven using a 0.2-Hz sine wave. This light, repeatable stimulus could be directed to contact a precise location at regular intervals.

The first experiment contained two treatments. In one, I positioned the experimental disturbance such that one or two nymphs were lightly brushed every 5 s. In the other, the brush lightly contacted the host plant stem every 5 s, at approximately the same distance from the female as were the contacted nymphs. Each treatment continued for 5 min, preceded by a 5-min pre-stimulus period to establish baseline levels of activity. I recorded signals using an accelerometer attached to the stem below the aggregation. The response variables were, for nymphs, the number of group signals produced, and, for females, the distance moved. Group signals are distinctive events that can be monitored both acoustically and visually (see Results). Because females normally are stationary at the base of the aggregation, then walk toward or over the aggregation to defend it, their movements provide an index of their antipredator response. For statistical analysis, I subtracted the levels of female movement and nymphal signaling during pre-stimulus periods (when non-zero) from levels observed during stimulus presentations. I then compared the adjusted levels during the two treatments using a Wilcoxon signed-rank test. In this experiment (and in the following two), I presented treatments to a given aggregation 35-45 min apart, alternating treatment order between subjects to control for order effects.

Importance of substrate-borne vibration

The vibrational signals recorded from disturbed nymphs might not be necessary to recruit the mother's response, if nymphal signals also are transmitted as airborne sounds or if chemical or visual cues are produced along with vibrational signals (see Wood, 1976). To test whether vibrational signals are necessary for evoking the female's response, I stimulated nymphal aggregations to signal using the experimental disturbance described above. In each of two treatments, nymphs were lightly brushed once every 2 s for two minutes. However, in one treatment, I severed the vibrational channel by cutting out a short segment of stem, *ca* 5 mm long, between the aggregation and the mother's position below it (the mother was removed from the plant during this procedure, then replaced). In the other treatment, I maintained the vibrational channel by replacing the excised stem segment and securing it with glue-stick adhesive. I cut out and reattached the stem segment before conducting the experiment, and thus the adhesive and the plant manipulation were present in both treatments regardless of order. I supported the distal portion of the stem in its original position using adjustable clamps mounted on a tripod. I scored the mother's response as positive if she moved toward the aggregation, and negative if she remained stationary. Mothers were approximately 2 cm away from the aggregation in both treatments, within the range of female locations documented in the field (R. Cocroft, unpub. data). The gap in the stem was located adjacent to the nymphs, so that females could move forward 1-2 cm

before reaching the gap. I compared female responses in the two treatments using a sign test.

Although vibrational signals will be transmitted between the nymphs and the mother when the excised stem segment is replaced, this manipulation of the host plant is likely to alter the response characteristics of the transmission channel. However, alterations in the signals caused by the experimental procedure should only have the effect of biasing females against responding at all, and do not alter interpretation of experimental results in the event of a positive response.

Importance of synchronization in signaling

Previous results showed that mothers responded to signals that were coordinated into group displays, but not to the same signals presented in random temporal patterns (Cocroft, 1996). To determine whether this response is also characteristic of nymphs, I conducted a playback experiment to nymphal aggregations from which the mother was temporarily removed. I constructed playback stimuli from individual nymphal signals obtained from the onset of group signals or from occasional, spontaneous signaling by individuals. I selected ten signals that differed in details of amplitude-time structure and thus presumably came from different individuals. I digitally combined these signals in two temporal patterns (see Cocroft, 1996). In the first, I placed signals randomly with respect to each other within a 3-s window, repeating this pattern for 1 min. In the second, I combined the same signals into a group signal (as in natural nymphal displays), repeating it every 3 s for 1 min (see Fig. 3, upper panel).

Nymphs make characteristic and distinctive movements when they signal, rocking rapidly to one side and back and lifting one tarsus from the substrate. These movements are easily detected in close-up video, and provide a visual assay of signaling behavior. Because, in this experiment, one of the playback stimuli itself included group signals, I used both visual and acoustic monitoring to score the production of group signals by nymphs.

Signaling thresholds

In communication systems that mediate responses to changing features of the environment, the level of stimulus required before an individual will signal can change according to context (Fitzgerald, 1988; Seeley, 1994). As a first step in exploring the influence of experience on nymphal signaling behavior, I asked whether the signaling thresholds of nymphs would be altered if they had recently experienced a predation event. In particular, because predator attacks can be clumped in time (Cocroft, 1998), nymphs might be expected to have a lower threshold of signaling after a recent experience of predation. The finding that nymphs will signal in response to signals from their siblings (Cocroft, 1996) suggested that signal playback could provide a quantitative, repeatable stimulus for examining signaling thresholds.

Determining absolute signaling thresholds (defined here as the minimum stimulus level required to evoke group signaling) for a particular family group at a particular stage of development would be problematic, because it would require a randomized presentation of different stimulus levels, separated by time intervals deemed sufficient (*a priori*) to restore thresholds to 'resting' levels. Use of short intervals might be likely to change the level of the threshold over the course of the experiment, while use of longer intervals would result

in an experiment conducted over a long enough time that thresholds might change because of circadian, ontogenetic, or other factors.

However, if the goal is to determine whether thresholds change, and in what direction, then identifying such *relative* changes is more straightforward. The approach I used was to construct a ramped series of stimuli (1 signal, then 2 synchronized signals, then 3 synchronized signals, *etc.*; see below for details) and present the same series to a family group in two different contexts. In one treatment, I left the aggregation as undisturbed as possible before the stimulus series was presented. In the second treatment I used light, repetitive contact with a brush (every 2 s for 20 s) to simulate the approach of a predator and thereby elicit synchronized, group signaling. I then waited until nymphs had ceased signaling for at least 10 s, then presented the stimulus series. To reduce the residual effect of the first treatment a family had received, I presented the second treatment the following day (*ca* 24 h later). However, because residual effects could not be ruled out, I alternated the order of treatments between families. I removed attending females by hand (as quickly and unobtrusively as possible) from their positions below the aggregation 1 h prior to each treatment, replacing them after the treatment was finished. Observations of nymphs during and after removal of the female suggested that this procedure did not cause nymphal signaling or dispersal.

To construct the playback stimuli, I first recorded eight individual signals from each aggregation. I then created a series of stimuli for each aggregation, containing from one to eight signals (Fig. 1a). For the stimuli with 2-5 signals, signals were separated by 60-80 ms, typical of the interval between signals in natural displays (see Results). For stimuli with more than 5 signals, each additional signal was placed within the series of 5 signals, resulting in the superposition of signals seen in natural group displays. I played each stimulus (consisting of N signals) 6 times at a rate of one every 2 s, within the range of repetition rates of natural group signals; the next stimulus ($N + 1$ signals) was begun after a 10-s pause (Fig. 1b).

As an (arbitrary) index of signaling threshold, I used the number of signals in the stimulus that first evoked synchronized signaling in five or more nymphs (*e.g.* a score of '4' would indicate that the first stimulus to evoke signaling by five or more nymphs contained four individual signals). I scored nymphal signaling behavior from videotape records on the basis of the movements associated with signal production. Because group signals oc-

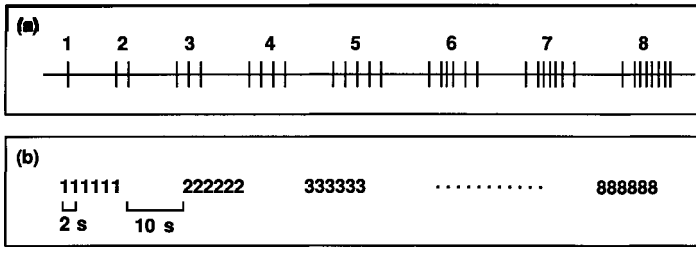

Fig. 1. Design of the playback stimuli used to assess changes in nymphal signaling thresholds. (a) Stimuli consisted of increasing numbers of individual nymphal signals, ranging from one to eight. (b) Each stimulus (N signals) was played six times, at a rate of one every two s; the next stimulus ($N + 1$) was played after a 10 s pause.

cur as signaling sweeps across an aggregation, and are separated by pauses, I considered nymphs to have signaled synchronously if they participated in the same coordinated burst of signals. Close-up videotapes contained a record of the behavior of 9-20 nymphs within an aggregation, with the same number of nymphs in the same portion of the aggregation scored in each treatment. I quantified the signaling behavior of each nymph by replaying the video and scoring whether or not it signaled in response to each of the playback stimuli. The number of signals required to cause five or more nymphs to respond was compared between treatments using a Wilcoxon signed-rank test.

Results

Vibrational signals

Vibrational signals of nymphal *U. crassicornis* are shown in Fig. 2. Note the contrast between brief, individual nymphal signals (Fig. 2a, b) and group signals produced by many individuals within an aggregation (Fig. 2b, c). Group signals were distinctive visually as well as acoustically, because the characteristic movements of signaling nymphs create the effect of a wave as signaling travels rapidly across an aggregation. Each signaling nymph appeared to signal only once during a group signal.

An individual signal typically consisted of a brief series of pulses, lasting 30-40 ms (35.9 ± 9.2 ms [values are reported as $\bar{x} \pm$ SD]; $N = 25$ aggregations, 5 signals each). Pulse repetition rate was variable, averaging 548.3 ± 208.1 per s; in some signals, discrete pulses were not clearly distinguishable. The interval between individual signals during group displays (measured at the beginning of the display, when individual signals are still distinct because only a few individuals have begun to signal) was 70.0 ± 14.4 ms.

Composite, group signals formed by the superposition of individual signals had an average duration of 520 ± 85 ms ($N = 25$ aggregations, 5 signals each). Group signals were produced in a series by disturbed aggregations, at rates of one every s or more (Fig. 2c). Group signals had a characteristic amplitude-time envelope, presumably reflecting the number of individuals signaling at any one time. The point of greatest amplitude in group signals was near the midpoint; dividing the time of maximum amplitude by the duration of the signal yielded a value of 0.47 ± 0.08.

The frequency profile of a vibrational signal will be affected by the properties of the particular substrate on which it is recorded (*e.g.* Michel-

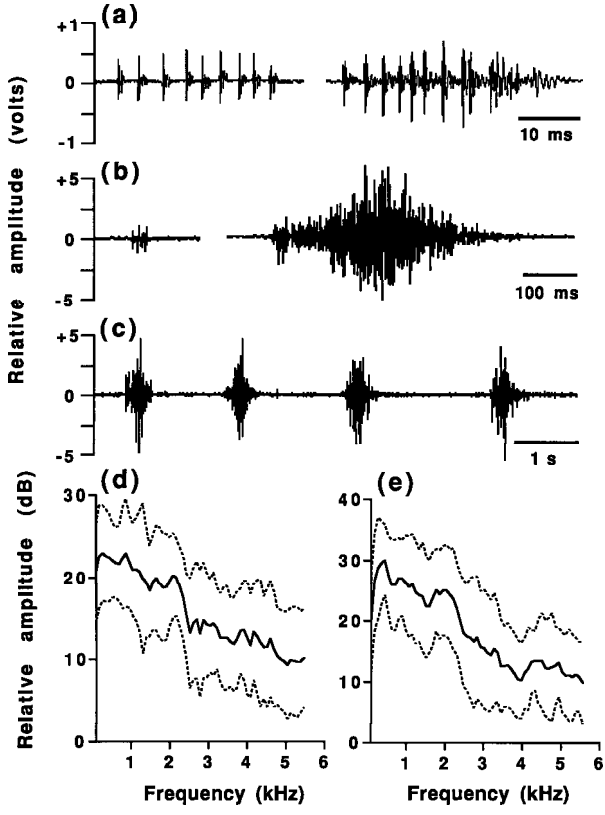

Fig. 2. Waveforms of vibrational signals of nymphal *U. crassicornis*. (a) Two individual signals; (b) An individual signal and a coordinated, group signal from an aggregation containing 37 3rd- and 4th-instar nymphs; (c) A series of group signals from the same aggregation as in [b]; (d) Spectrum ($\bar{x} \pm$ SD) of individual signals, one each from 25 aggregations; (e) Spectrum ($\bar{x} \pm$ SD) of group signals, one each from 25 aggregations. All signals were recorded at 27 + 1°C.

sen *et al.*, 1982; Barth *et al.*, 1988). However, aggregations in the lab occurred on the same host plant species, and on the same part of the plant, as aggregations in the field (insects in the lab freely chose oviposition and aggregation sites). The substrates encountered in the lab are thus typical of natural substrates. The frequency spectra of individual and group signals were similar and contained a broad range of frequencies (Fig. 2d, e). For group signals, which contained a large sample of individual signals, the frequency with the greatest amplitude was 502 ± 373 Hz.

Offspring signaling and maternal response evoked by disturbance

Nymphal signaling behavior can be evoked by an experimental disturbance simulating the approach of a predator (Fig. 3a). When one or two nymphs in an aggregation were contacted lightly and repeatedly with a brush, a series of group signals was produced by many individuals within the aggregation (number of group signals in 5 minutes: 38.2 ± 18.0, $N = 12$ aggregations). After group signaling was initiated, it often was the case that each new contact evoked one group signal. In contrast, when the host plant stem was contacted with a brush, nymphs usually remained silent (0.7 ± 1.1 group signals in 5 min, $N = 12$ aggregations; Wilcoxon signed-ranks test: $z = 3.06$, $N = 12$, $p < 0.01$). Nymphs seldom produced group signals during prestimulus periods (1.0 ± 1.8 group signals in 5 min, $N = 12$ aggregations).

Maternal behavior also differed strikingly between the two treatments (Fig. 3b). When the disturbance contacted the nymphs, 9 out of 12 mothers approached the offspring aggregation, often walking back and forth over it several times (distance moved: 12.4 ± 12.3 cm, $N = 12$); five mothers also began wing fanning, and three approached and repeatedly kicked the experimental stimulus. When the disturbance contacted the plant stem, mothers remained stationary at the base of the aggregation (distance moved: 0.2 ± 0.6 cm, $N = 12$; Wilcoxon signed-ranks test: $z = 2.67$, $N = 12$ with 3 ties, $p < 0.01$), as they did during pre-stimulus periods. The distance between the stimulus and the mother was similar in the two treatments

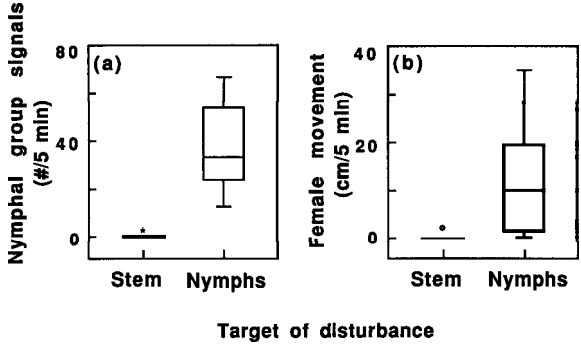

Fig. 3. Responses of *U. crassicornis* families to disturbance (light contact) of the plant stem and of the nymphs themselves. (a) Nymphal group signaling; (b) Distance moved by mothers as they walked onto the aggregation from a stationary position below it.

(nymphal contact: 16.2 ± 5.7 cm; branch contact: 16.4 ± 5.5 cm; Wilcoxon signed-ranks test, $z = -0.08$, $N = 12$, NS). Mothers thus appeared to engage in brood defense not in response to the experimental disturbance per se but in response to the behavior of the nymphs.

Dependence of communication on the vibrational channel

Communication between mother and offspring is dependent on the presence of a continuous vibrational channel. When a short section of stem was removed between the nymphs and the mother, blocking the transmission of vibrational signals, mothers did not respond when nymphs produced group signals. When the stem section was replaced, restoring the transmission of vibrational signals, all mothers approached the aggregation when nymphs signaled (Sign test: two tailed, $N = 12$, $p < 0.001$). Six mothers also began wing fanning, and six kicked the experimental stimulus as it neared the nymphs. The number of nymphal group signals evoked did not differ between the two (2-min) treatments (stem continuous: 51.9 ± 8.1 signals; stem with gap: 51.2 ± 4.9 signals; Wilcoxon signed-rank test: $z = -0.59$, $N = 12$ aggregations, NS).

Temporal coordination of signaling

Temporal coordination of signals is required to evoke signaling by otherwise undisturbed nymphs (Fig. 4). Playback of individual signals arranged in random patterns evoked a series of group signals in only 1 out of 12 aggregations (average rate of group signaling: 2.3 ± 7.7 per min.). In contrast, playback of the same signals arranged in a coordinated pattern evoked a series of group signals from all 12 aggregations (average rate of group signaling: 15.8 ± 6.6/min; Wilcoxon signed-ranks test: $z = 2.67$, $N = 12$ aggregations, $p < 0.01$).

Response thresholds of nymphal aggregations

Signaling thresholds within nymphal aggregations are modulated as a result of recent experience (Fig. 5). In response to a ramped series of signals, five or more nymphs in otherwise undisturbed aggregations began coordinated signaling when the number of signals in the stimulus ranged from three to eight (4.8 ± 1.9 signals). Note that this is not an absolute threshold because it

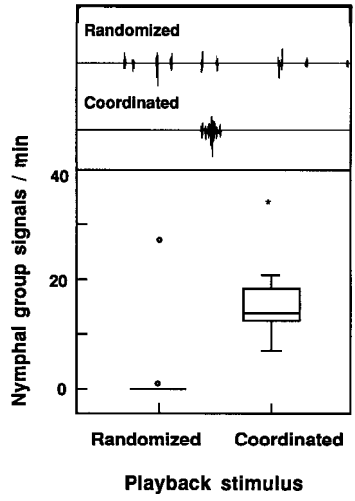

Fig. 4. Responses of *U. crassicornis* nymphs to playback of individual signals from their own aggregation, arranged in two temporal patterns (representative playback stimuli shown in upper panel). Measure shown is the number of group signals produced by aggregations during 1-min playback of each stimulus.

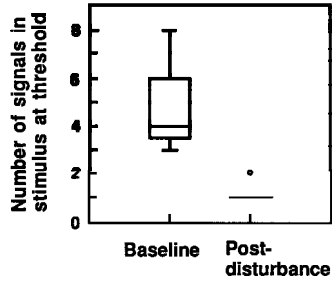

Fig. 5. Relative signaling thresholds of nymphal aggregations when undisturbed and after a simulated predation event. Y-axis shows the point in a ramped series of stimuli at which five or more nymphs began signaling synchronously.

may have been influenced by the signals that individuals perceived before responding. In contrast, after nymphs had been disturbed by simulated predation (and had been producing group signals), five or more nymphs in most aggregations began coordinated signaling in response to the signal of a single individual (1.2 ± 0.4 signals). This difference was highly significant (Wilcoxon signed-ranks test: $z = -2.97$, $N = 11$, $p < 0.01$). Note that

playbacks were begun only after nymphs had ceased signaling in response to the experimental disturbance.

Limited evidence suggests that the change in threshold was temporary, with levels returning to baseline by the next day. Thesholds for undisturbed nymphs did not differ depending on whether the 'predation' treatment occurred the day after (5.0 ± 2.0 signals, $N = 6$ aggregations) or the day before (4.6 ± 1.9 signals, $N = 5$ aggregations; t-test, $t = 0.33$, df $= 9$, NS).

Discussion

Signals that recruit the mother's antipredator behavior are an important component of maternal care in *U. crassicornis*. Maternal protection is essential for nymphal survival because predation, especially by invertebrates, is intense in the field (Wood, 1976; Dowell & Johnson, 1986; Cocroft, 1996, 1998). The mother's active defense is the nymphs' main protection once attacked (Brach, 1975; Wood, 1975; Dowell & Johnson, 1986; Cocroft, 1998). This study (and Cocroft, 1996) shows that the substrate-borne vibrational signals of nymphs are used both in soliciting maternal defense and in communicating within the brood.

Production of group signals in the laboratory was context-dependent, occurring only when nymphs were disturbed by a simulated predator. This result mirrors the behavior of nymphs of *U. crassicornis* (and of the closely related *U. spinosa*) in the field. Under natural conditions, undisturbed nymphs are silent or produce occasional individual signals; when a predator is present, nymphs produce a series of group signals (Cocroft, 1996, in press). In both the laboratory and the field, nymphs continued to signal as long as the disturbance was present, suggesting that signals may function not only in initially alerting the mother, but also in communicating with her throughout the predator's attack. And, as in the field, nymphal signaling in the laboratory was associated with active defense by the female, including approach, wingbuzzing, and kicking.

Vibrational signals appear to be the only means of antipredator communication between nymphal offspring and their mother, at least in the absence of injury to the nymphs (see Wood, 1976). When transmission of vibrational signals was blocked between mother and offspring, mothers

showed no response to nymphal signals. If there is any airborne energy in the signals (which are inaudible to a human observer), or if nymphal signaling behavior also provides visual or chemical cues, these are insufficient to evoke a maternal response.

One of the most striking aspects of the signaling behavior of *U. crassicornis* nymphs is that individual signals are synchronized, resulting in the production of coordinated, group signals. In this study, contact of one or a few nymphs with an experimental disturbance triggered signaling by many individuals within the aggregation. Playback experiments (Cocroft, 1996, this study) show that nymphs will signal in an immediate response to signals of their siblings, and this is evidently how coordinated signals are generated. This temporal coordination among signalers is important in communication. Previous results (Cocroft, 1996) showed that mothers only responded to signals of individual nymphs when they were synchronized, and not when they occurred in random temporal patterns. This study showed that nymphs in undisturbed aggregations only signaled in response to coordinated signals from multiple individuals. Thus both sets of signal receivers within a family group — the mother and other, non-signaling nymphs — require synchronization of signals before responding.

Why do mothers and non-signaling nymphs respond only to synchronized signals? At the ultimate level, if there is a cost to offspring defense (*e.g.* energetic costs or an increase in the mother's conspicuousness to predators capable of removing her), mothers and offspring may be selected to respond only when several individuals indicate shared perception of a common threat. At the proximate level, the greater intensity and duration of group signals may increase conspicuousness in the presence of noise produced by wind, other signaling species, or a predator's approach. However, this does not explain why receivers do not respond to individual signals even in the absence of such noise, as seen in laboratory playbacks. It is also possible that signals given individually have a different communicative function. Sporadic, individual signals are common in undisturbed aggregations in the laboratory and in the field (Cocroft, in press), but their role in this communication system is unclear.

Does coordination among signaling nymphs represents cooperation? Coordination among close neighbors may constitute a mutualism (Mesterton-Gibbons & Dugatkin, 1992) if neighbors within a given area are all at

risk from a predator and can all be protected by the mother. However, signaling interactions between nymphs could be competitive, if there is a potential for differential access to the protection afforded by the mother (West-Eberhard, 1984; Lazarus & Inglis, 1986). Aggregations of late-instar nymphs can extend for many female body lengths along the plant stem, and *U. crassicornis* mothers appear unable to defend all offspring simultaneously (Cocroft, 1998; also see Windsor, 1987, for a parallel example in a subsocial beetle). There also is differential predation risk for individuals as a function of their position in the aggregation, with, for example, individuals on the margins being more vulnerable (Cocroft, 1998). Such a differential in predation risk could create conflicts of interest (Hamilton, 1971), especially if responding to nymphal signals increases the mother's predation risk, or if her movement towards some nymphs renders other nymphs more vulnerable. It is not clear whether nymphs in different locations can, by signaling, influence the female's precise position within the aggregation. If so, signals would provide a mechanism by which nymphs could compete. It is also possible that non-cooperation could be manifested by a failure to signal when more distant nymphs are threatened.

Nymphal signaling thresholds change as a result of experience. Signaling by undisturbed nymphs was only triggered by playback of several, coordinated individual signals. In contrast, after a recent experience of (simulated) predation, comparable levels of signaling could be initiated by playback of only one signal. These results suggest that, when a predator first appears, group signaling is triggered only when several individuals signal together, but that after a predator has been present for some time, group signaling can be triggered by a single individual. Nymphs signal not only in response to the behavior of other nymphs, but also in response to contact (this study) or the proximity of a predator (Cocroft, in press); however, changes in response thresholds to these stimuli have not been investigated. The apparent sensitization (Petrinovich, 1984) of nymphal responses after a predator encounter raises the question of why response thresholds in undisturbed nymphal aggregations are higher than the minimum, and suggests the existence of costs to signaling in the absence of a predator (*e.g.* engaging in defensive behavior may make the female more conspicuous to other predators in the environment).

What aspects of the ecology of these treehoppers might have selected for offspring communication about predators, as opposed to female vigilance alone? One potential benefit of group living is enhanced detection of predators (Kenward, 1978; Bertram, 1980). Because the nymphs far outnumber the female, they may be more likely to detect a predator. Furthermore, nymphal aggregations occupy up to 15 cm of host plant stem, and females may be 2-4 cm away from the nearest nymph (R. Cocroft, unpub. data). Individuals on the opposite side of the stem, the far end of the aggregation, or behind leaves will be outside the female's visual field and up to 10 body lengths away. These offspring may be better able than the mother to assess their current predation risk.

Wood (1976) showed that chemical cues associated with injured *U. crassicornis* nymphs can elicit the mother's defensive behavior. When a disk of filter paper with extract from a crushed nymph was placed near an aggregation, the female responded as if a predator were present (see also Nault *et al.*, 1974). The present study does not address the role of chemicals released by injury. Instead, it demonstrates that in the absence of injury to the nymphs, alarm signaling is limited to the vibrational channel. It is possible that injured nymphs release a pheromonal alarm signal as do aphids (Nault & Montgomery, 1977). Alternatively, females may detect cues associated with nymphal injury even if nymphs do not produce a specially-evolved signal (*cf.* Williams, 1992). It is also unknown whether nymphs in Wood's (1976) study produced vibrational signals in response to the chemical cues from an injured nymph. In any case, chemicals released by injured nymphs provide females (and perhaps nymphs) with a third source of information about predators, in addition to nymphal vibrational signals and direct perception. Wood (1976) suggested that, because chemical cues that require a rapid response are most effective over short distances, such cues in *U. crassicornis* are likely to be most effective in 1st-instar aggregations, which are only 2-3 cm long. First-instar nymphs apparently do not produce vibrational signals (pers. obs.), and thus there may be a developmental shift in the cues used by mothers to detect predation of their offspring.

Additional defenses are involved in protection against vertebrate predators in *U. crassicornis*. Aposematism has been suggested as a function of the teneral adult coloration (green, yellow and red; Wood, 1975, 1977)

and may apply equally to nymphal coloration, at least in later instars where nymphs are red, white and black. Teneral adults and 5th-instar nymphs were rejected in feeding tests using lizards (*Anolis carolinensis*; Wood, 1977), which treated them as if they were distasteful. Interactions of tending females with vertebrate predators have not been observed, but the female's defensive behaviors are unlikely to be as effective against lizards or birds as they are against relatively small invertebrates.

Studies of vibrational communication in *U. crassicornis* (Cocroft, 1996, in press, this study) provide the first detailed examination of offspring-parent communication in a subsocial insect. As in vertebrate family groups, communication is involved in soliciting parental resources — in this case protection against predators. A unique feature of communication in *Umbonia* is that mothers only respond when multiple individuals signal together. This coordination has the earmarks of cooperation, since individuals together can accomplish what separately they cannot. However, there may also be an opportunity for competition among signaling individuals for the mother's proximity. Because the responses of the receiver set the ground rules for communication, only a more detailed characterization of the mother's response to signals can settle this question. In any event, evidence is growing for the importance of communication within *Umbonia* aggregations. Thus far documented are nymphal vibrational signals that elicit defense (Cocroft, 1996, in press, this study); maternal vibrational signals after predation events (Cocroft, in press); maternal tactile signals that keep nymphs within the aggregation (Wood, 1974); and maternal alarm responses mediated by chemical cues (Wood, 1976). Taken together, these communication systems recall in complexity those of the eusocial insects, and suggest a central role for communication in the biology of these insect family groups.

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