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Offspring chemical cues affect maternal food provisioning in burrower bugs, *Sehirus cinctus*

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Female burrower bugs (*Sehirus cinctus*, Hemiptera: Cydnidae) show extended care for their offspring. They guard their clutch and feed the hatched nymphs up to the third larval instar. Previous research indicated that nymphs partly regulate maternal food provisioning, but how nymphs accomplish this is unknown. We tested the hypothesis that nymphs solicit maternal provisioning by condition-dependent chemical signalling and we postulated the existence of a solicitation pheromone. Clutches of 30 nymphs were hand-reared in either low- or high-food conditions. After moult to second instar, cuticular compounds were extracted in hexane. An independent set of test mothers caring for offspring were subsequently exposed to extracts of nymphs from either the low- or the high-food treatment. Two control groups were also involved, one exposed to the solvent hexane and one with no treatment. As predicted for a solicitation pheromone, test mothers exposed to extracts from nymphs reared under low food provisioned more than those exposed to extracts from nymphs reared under high-food treatment. Contrary to our expectation, however, nymph extracts had an overall inhibiting effect on maternal provisioning. The effects of extract exposure on maternal provisioning were short-lasting, suggesting that the critical cues may be volatile. Our results suggest complex chemical communication in burrower bug families for the short-term regulation of maternal provisioning, potentially involving both provisioning-releasing solicitation pheromones and inhibiting chemical cues.

The regulation of parental care often involves behaviours and traits expressed in offspring that may reflect the offspring's demand or need for parental care (Hussell 1988; Kölliker 2003). The evolutionary driving forces underlying these often conspicuous solicitation traits are thought to include sibling rivalry, parent–offspring conflict (Trivers 1974; Godfray 1995; Mock & Parker 1997; Parker et al. 2002b), and the coadaptation of solicitation traits and parental provisioning strategies (Cheverud & Moore 1994; Wolf & Brodie 1998; Agrawal et al. 2001; Kölliker 2005). Models for the evolutionary resolution of family conflicts predict that solicitation traits, and the corresponding provisioning by parents, should increase in intensity with 'need', either because of selection on parents to maximize fitness returns on investment (Godfray 1991), or because of selection on offspring to outcompete siblings for parental resources (Parker et al. 2002a).

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Solicitation traits have been best studied in birds (Kilner & Johnstone 1997; Mock & Parker 1997; Wright & Leonard 2002). The highly conspicuous begging display performed by many altricial bird nestlings plays an integral role in the regulation and evolution of parental provisioning (Kilner & Johnstone 1997; Kölliker et al. 2000; Budden & Wright 2001; Kölliker & Richner 2001; Royle et al. 2002; Wright & Leonard 2002). Parental care involving the physical interaction with progeny, which is a prerequisite for solicitation traits to evolve, is less widespread in arthropods (Clutton-Brock 1991). Among those species, mostly females show extended forms of care including the guarding of eggs, and the protection and provisioning of hatched progeny (Tallamy 1984, 2001).

Few studies have addressed the role of offspring solicitation traits in the regulation of parental (or worker in the case of eusocial insects) care in arthropods (Kilner & Johnstone 1997). Those that have reveal a diversity of signal modalities, but have not shown chemical signals to be involved in offspring influence on parental provisioning.

Coelotes terrestris spiderlings may stimulate maternal provisioning by tactile signals (i.e. stroking their mother's

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chelicerae with their palps; Gundelmann et al. 1988). Nymphs of the spatially aggregated clutches of treehopper *Umbonia crassicornis* produce, when agitated, group-coordinated substrate-born vibrational signals to induce maternal protection against potential predators (Cocroft 1999). In eusocial insects, larvae may influence within-colony allocation of food by workers by head movements and mandible flexing (Hölldobler & Wilson 1990; but see Cassill & Tschinkel 1995). In wasps, larvae solicit food by rubbing their mandibles against their cell wall and thereby produce rhythmic acoustic signals (Ishay & Landau 1972). Burying beetle (*Nicrophorus* spp.) larvae perform a postural begging display by raising their head and waving their legs towards the parent, which affects parental trophallaxis (Rauter & Moore 1999; Smiseth & Moore 2002; Smiseth et al. 2003). Finally, the hunger state of individual larvae influences food allocation by workers inside colonies of fire ants, *Solenopsis invicta* (Cassill & Tschinkel 1995) and honeybees, *Apis mellifera* (Huang & Otis 1991). Although no solicitation behaviours/traits were investigated in these two studies, the authors of both hypothesized that larval chemical cues might be involved (Huang & Otis 1991; Cassill & Tschinkel 1995). The only example in an arthropod species of a chemical signal regulating parental/worker provisioning is the honeybee 'brood pheromone', a blend of 10 fatty acid esters on the bee larval cuticle (see Le Conte et al. 2001; Blomqvist & Howard 2003 and references therein). Brood pheromone increases the foraging activity of the colony by acting both as a releaser for pollen foraging by existing forager workers (Pankiw et al. 1998; Barron et al. 2002) and/or as a primer altering the age of worker transition to foragers (Le Conte et al. 2001; Pankiw & Page 2001).

Burrower bug, *Sehirus cinctus*, mothers care for their progeny by guarding their eggs, and guarding and food provisioning their nymphs up to the third larval instar (Sites & McPherson 1982; Kight 1997; Agrawal et al. 2001). They bring the mint (*Lamium purpureum*) nutlets to the nymphs, which live gregariously under cover or in a burrow (Sites & McPherson 1982). Food is not allocated to individual bug nymphs, but rather deposited in the vicinity of the nymphs, which then independently forage on the provisioned nutlets (Sites & McPherson 1982; Kight 1997; Agrawal et al. 2001). Unrelated females may breed in close spatial proximity in the wild (often <6 cm apart; Agrawal et al. 2004), and whole clutches of nymphs sometimes leave their shelter to join other clutches. Once dispersed they may be provisioned by unrelated females (Agrawal et al. 2004), or may forage on their own.

Prior studies suggest that offspring at least partially regulate maternal provisioning in *S. cinctus*. Mothers partly adjust their care to the developmental state of their progeny (Kight 1997). In a recent cross-foster experiment, maternal provisioning correlated positively with the size of the unrelated foster clutch, and nymph siblings shared a capacity to elicit maternal provisioning with a likely genetic basis (Agrawal et al. 2001). Mothers also increased provisioning when the provisioned food was continuously removed by the experimenters (Agrawal et al. 2001), suggesting that a condition-dependent nymph signal may be involved, although maternal gauging of food could be an alternative explanation (Agrawal et al. 2001). It is not known what traits or signals are responsible for this influence on maternal effort by nymphs.

In this study we experimentally tested the hypothesis that nymph chemical cues are involved in the regulation of maternal provisioning in *S. cinctus*. By analogy to solicitation displays in altricial birds, and based on the indirect evidence presented above, we postulated a solicitation pheromone and specifically predicted that exposure to cuticular extracts from nymphs reared under low food would induce higher maternal provisioning than extracts from nymphs reared under high food. Using a form of chemical 'playback' experiment, we exposed attendant mothers to signals from unrelated clutches of manipulated condition and conducted a bioassay of maternal provisioning to determine whether chemical signals are at play in this behavioural interaction.

METHODS

We collected adult *S. cinctus* in mid-April 2003 in Bloomington, Indiana, U.S.A. by searching fields of the mint *Lamium purpureum* (Labiatae) (Sites & McPherson 1982). In the laboratory, we initially set up all adult bugs in individual housing units. Each unit consisted of a 100×15 mm polystyrene petri dish, playsand that was thoroughly wetted with room temperature tap water, and two to four *L. purpureum* nutlets placed in the centre of the dish. All dishes were checked daily for egg deposition and watered as needed. Nutlets were removed and replaced every 2 days. Upon deposition of full-sib clutches (Brown et al. 2003), all nutlets were removed from the dish. The female bugs guarding their clutches were held in a Percival Scientific incubator on a 13:11 h, 24:18°C light:dark cycle. Rotation of housing units occurred daily, such that each petri dish's position was varied within its own petri rack from top to bottom, and all racks shifted position within the incubator with respect to horizontal and vertical position.

Hand Rearing of Nymphs and Extraction of Cuticular Compounds

We used the first 45 clutches laid for extraction of cuticular compounds. On the day of hatching, we sedated clutches on ice for at least 45 min to reduce nymph activity and allow reliable counts of clutch size. Each clutch was then split into two groups of 30 haphazardly chosen nymphs. Each half-clutch was set up and housed individually in a new petri dish (polystyrene, 150×15 mm). We assigned one randomly chosen half-clutch to the high-food treatment, and the other half-clutch to the low-food treatment (see below). To serve as a shelter, we cut a 20-mm-long piece of plastic tubing into thirds and placed one-third in each petri dish. To the concave portion of this shelter we firmly attached a roughly fitting piece of filter paper using a soldering iron. This filter paper lining served no purpose in the extraction clutches except to keep the rearing environment identical to the environment of the test clutches later exposed to the extracts (see below).

Beginning the day after emergence, extraction clutches were hand-fed daily with mint nutlets. To ensure that our food levels in the high- and low-food treatments remained within the natural range of maternal provisioning, we derived the daily number of nutlets provided from maternal provisioning rates acquired in past experiments (Agrawal et al. 2001). The amount of food in the high-food treatment was taken to correspond to the 75th percentile and that in the low-food treatment to the 25th percentile of the daily maternal provisioning distributions. Heavily moulded nutlets were removed each day.

Once all nymphs from the high- and low-food treatments of a given extraction family reached the second instar, we carried out the extraction of the putative cuticular compounds. Because some mortality occurred during the first larval instar, the number of nymphs for extraction was reduced from the original 30 per family to an average $\pm$ SD of 27.87 ± 1.72 in the high-food treatment and 27.31 ± 2.14 in the low-food treatment (Wilcoxon two-sample test; $Z = 1.11$, $N = 78$, $P = 0.268$). After initial cold sedation as above, we transferred the nymphs to culture tubes and immediately put them back on ice. We subsequently added 0.5 ml of hexane (99% pure; Sigma-Aldrich, St Louis, Missouri, U.S.A.) to the culture tube for 10 min. The hexane extract was then decanted into an autosampler vial (3.7 ml, Fisherbrand, Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.) and stored at $-30\,^{\circ}C$ until used in the extract exposure experiments (see below). The median storage time of extracts before use in the exposure experiments was 3.5 days (range 1–24 days).

Bioassay: Extract Exposure Experiments

We used a separate set of test clutches in the extract exposure experiments. These clutches were randomly assigned to four experimental groups on the day of egg laying: C1, no exposure (filter paper control); C2, exposure to the solvent hexane (hexane control); EXT-H, exposure to extracts from nymphs in the high-food treatment, and EXT-L, exposure to extracts from nymphs in the low-food treatment. We assigned the first clutch to a treatment by use of a random number generator. A random sequence of treatments was established similarly, and every subsequent clutch was assigned in an alternating fashion cycling through this sequence.

To keep other stimuli from nymphs as constant as possible among experimental replicates, we culled test clutches to 50 nymphs each on the first day after hatching. These 50 nymphs remained with their mothers. Three 32-mm bottle caps served as sources of mint nutlets and were placed at approximately the nine, twelve, and three o'clock positions with respect to the filter-paper-lined plastic shelter (Agrawal et al. 2001). We minimized variation in the condition of the test nymphs before the extract exposure experiments by placing two nutlets directly under the shelter with the nymphs. To provide mothers the opportunity to forage and find food in the food caps, but still avoid variation in maternal provisioning to strongly influence nutritional condition of nymphs, we placed one nutlet in each of the three food caps.

The extract exposure experiments were carried out on the second day after hatching, starting at approximately 0900 hours, the time maternal provisioning typically begins under laboratory conditions (A. F. Agrawal & E. D. Brodie III, unpublished data). Prior to exposure to extracts, test families were partially sedated in a $5\,^{\circ}C$ refrigerator for 15 min. To ensure full exposure of bug mothers and clutches, all individuals were gently put directly under the shelter. Each nutlet cap from the initial set-up was replaced with a new cap containing 20 nutlets each. The original shelter was then replaced by a new shelter lined with filter paper with one of the four treatments: filter paper only (treatment C1), 300 µl of the solvent hexane (treatment C2), 300 µl of cuticular extract from nymphs reared under high food (treatment EXT-H), or 300 µl of cuticular extract from nymphs reared under low food (treatment EXT-L). The filter paper was allowed to dry before introducing the shelter to the test clutch. Each day, four test clutches were randomly selected for video recording of maternal behaviour during the first 2 h of the experiments. The experimental room had a temperature range of $22–27\,^{\circ}C$ and a humidity level of approximately 80%. During the first 2 h of the experiments in the morning the mean $\pm$ SD temperature was $25 \pm 0.1\,^{\circ}C$ and during the last 2 h in the late afternoon it was $27 \pm 0.1\,^{\circ}C$.

Data Collection

We collected the provisioning data at 2-h increments over the next 8 h by counting the number of nutlets remaining in the three food caps. Provisioning was calculated as the difference between the number of nutlets initially offered (i.e. 3×20 nutlets) and the nutlets remaining (Agrawal et al. 2001). Nutlets that had been moved outside the cap but were not in the immediate vicinity of the shelter or progeny were not scored as provisioned. At every counting session the observer also noted whether the nymphs were staying inside the shelter or had dispersed. Dispersal was scored as such when the majority of the 50 nymphs were observed outside the hut. The observer scoring provisioning was blind with respect to the treatment assignments of clutches.

Analysis of Video Recordings: Maternal Time Budget

Video recordings were taken during the first 2 h of the extract exposure experiments. From the videos, we recorded the times when a female left the shelter, entered a food cap, left a food cap or entered the shelter. From these records, we calculated the time-budget variables (see Statistical Analyses below) that we hypothesized to be associated with the extract exposure treatment.

Statistical Analyses

Total sample size in the extract exposure experiments was 144 test clutches. Six females did not show provisioning at any time throughout nymphal development.

These clutches were excluded from any further analyses so that the net sample size for statistical analyses was 138 clutches (C1: $N = 21$; C2: $N = 39$; EXT-H: $N = 39$; EXT-L, $N = 39$). Provisioning rate was calculated and analysed separately for each of four 2-h increments of quantification. Because there was a certain time delay for some clutches between set up for extract exposure and the actual start of the first 2-h quantification period, we calculated provisioning rate during the first 2 h as nutlet-count/(120 + delay) × 120. The effect of our treatment on maternal provisioning was analysed using Poisson regression models as implemented in the generalized linear model (GENMOD) procedure of SAS (SAS 1999). Poisson regression was appropriate due to the count-nature and the positive skew of the provisioning data. Overdispersion of the data was corrected in the model by adjusting the parameter covariance matrix and the likelihood function by the scale parameter (i.e. the residual deviance divided by the residual degrees of freedom; see SAS 1999, User Manual). The effect of the extract exposure treatment on the incidence of nymph shelter leaving (shelter left: yes/no) was analysed by logistic regression models with binomial error and a logit-link using the LOGISTIC procedure (SAS 1999).

In the case of significant overall treatment effects, differences between individual treatment groups were tested by means of contrast analysis derived from the initial model (SAS 1999, User Manual).

The analysis of maternal time budgets was based on a subsample of 59 clutches for which video recordings were available. Our analyses focused on (1) the latency until the bug mother left the shelter for the first time, (2) the latency until she visited a food cap for the first time, and (3) the total time spent inside the shelter with the nymphs. The experimental effects of the extract exposure treatment on these behavioural variables were analysed using generalized linear models with normal errors (the GENMOD procedure, SAS 1999).

Based on our directional a priori prediction that maternal provisioning would be higher in the EXT-L than in the EXT-H treatment, we used a directional significance test according to Rice & Gaines (1994b) for testing this effect. Also, because the results from the provisioning analysis allowed us to predict an order for the treatment effects on the behavioural time-budget variables, we present for the time-budget analyses P values from both regular two-tailed tests and from ordered heterogeneity tests (Rice & Gaines 1994a). The P values corresponding to directional and ordered heterogeneity tests are denoted as P_{OHT}. All other P values correspond to regular two-tailed tests.

RESULTS

Maternal Food Provisioning

The extract exposure treatment had a significant overall effect on maternal provisioning during the first 2 h of exposure (Poisson regression: $F_{3,134} = 6.16$, $P < 0.001$; Fig. 1). There was a significant effect in the anticipated direction of food level under which extract nymphs were reared. Test mothers exposed to cuticular extracts from nymphs reared under low food provisioned significantly more than test mothers exposed to extracts from nymphs reared under high food (contrast EXT-H versus EXT-L: $F_{1,134} = 3.73$, $P_{OHT} = 0.035$; Fig. 1). Maternal provisioning was significantly reduced in clutches exposed to cuticular extracts compared with control clutches (contrast C1, C2 versus EXT-H, EXT-L: $F_{1,134} = 16.04$, $P < 0.001$; Fig. 1). The extract solvent hexane per se had no significant influence on maternal provisioning (contrast C1 versus C2: $F_{1,134} = 0.91$, $P = 0.343$; Fig. 1).

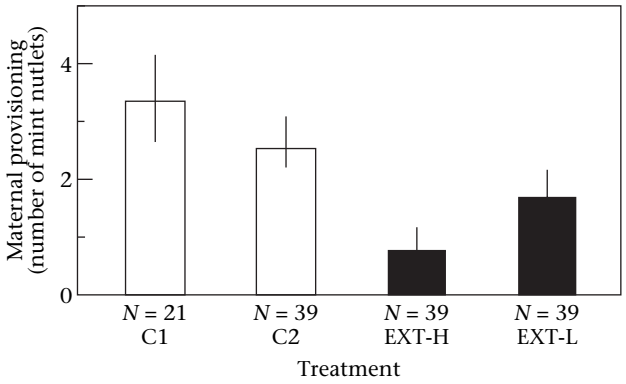

Figure 1. Effect of nymphal cuticular extracts on maternal food provisioning during the first 2 h of exposure. C1: filter paper control, C2: hexane control. Test mothers and test nymphs in group EXT-H were exposed to cuticular extracts from nymphs hand-reared under high food, while test mothers and nymphs in group EXT-L were exposed to cuticular extracts from nymphs hand-reared under low-food conditions. Shown are means and standard errors.

The effect of extract exposure on maternal provisioning was brief. No significant effect on provisioning was apparent 2–4 h ($F_{3,134} = 0.87$, $P = 0.458$), 4–6 h ($F_{3,134} = 1.98$, $P = 0.120$) or 6–8 h ($F_{3,134} = 0.71$, $P = 0.548$) after the start of the experiments. There was also no significant effect of the extract exposure treatment on maternal provisioning on the subsequent day ($F_{3,134} = 0.47$, $P = 0.703$).

Maternal Behaviour and Time Budget

None of the time-budget variables considered was significantly related to the extract exposure treatment in the available subsample (Table 1). The latency until the female left the shelter for the first time was the only variable showing a pattern consistent with the expectation from maternal provisioning (Fig. 1). To explain the treatment effect on maternal provisioning, this latency is expected to be longest in the EXT-H treatment, followed by the EXT-L, C2 and finally C1 treatment. Incorporating this expected order of effects into the significance test using ordered heterogeneity tests (Rice & Gaines 1994a) revealed a weak trend for the effect of the extract exposure on the latency to first maternal shelter leaving ($P < 0.10$; Table 1). There was also a weak though consistent negative correlation between maternal provisioning and this latency variable (Spearman rank correlation: $r_S = -0.21$, $N = 59$, $P_{OHT} = 0.068$).

Table 1. Effects of cuticular extract exposure on maternal time budget (units for all variables are given in minutes)

Variable	C1	C2	EXT-H	EXT-L	*F*	$P_{\text{two-tailed}}$ (P_{OHT})*
Latency to first shelter leaving ($N=59$)	41.12 (36.58)	45.61 (26.40)	59.14 (39.96)	47.48 (33.99)	0.72	0.546 (<0.10)
Latency to first visit of food cap ($N=46$)†	65.86 (35.05)	66.28 (25.83)	70.53 (33.12)	61.92 (29.15)	0.17	0.919 (~0.50)
Total time spent inside shelter ($N=59$)	94.76 (26.96)	76.16 (33.24)	86.25 (37.14)	87.73 (39.08)	0.64	0.596 (~0.70)

Shown are means ± SD for each treatment (C1: no exposure, filter paper control; C2: hexane control; EXT-H and EXT-L: exposure to extracts from nymphs in high- and low-food treatments, respectively), *F* values from generalized linear models and corresponding *P* values for two-tailed and ordered heterogeneity tests (OHT).
*Ordered heterogeneity test, according to Rice & Gaines (1994a). Expected order taken from results on maternal provisioning (Fig. 1).
†Female burrower bugs that never went to a food cap were not included for this measure.

Nymph Shelter Leaving

The extract exposure treatment also differentially affected the behaviour of the test nymphs. A disproportionately high incidence of shelter leaving occurred in the EXT-H compared with the EXT-L treatment. Shelter leaving was rare in the two control and the EXT-L treatments (Table 2). A full model analysing the effect of treatment on shelter leaving comparing all four treatment groups was performed on shelter leaving anytime during the 8 h of quantification. Shelter leaving occurred significantly more often in the EXT-H than in any of the other treatment groups (Table 2). On the day following the extract exposure experiments, seven of the 15 clutches that were found outside the shelter at the end of the experiments (see Table 2) had returned back under the shelter, while eight had remained outside.

DISCUSSION

In this study we have shown experimentally that chemical cues from the surface of nymphs are involved in the short-term regulation of maternal food provisioning and clutch behaviour in *S. cinctus*. Previous studies on chemical communication within animal families have focused on more static effects on progeny care, such as the recognition of own genetic offspring (reviewed in Wyatt 2003), the olfactory guidance of newborn mammals to their mother's mammae (Schaal et al. 2003) and the adjustment of worker activity and production to the number of larvae in bee hives based on brood pheromone (Pankiw et al. 1998; Pankiw & Page 2001; Barron et al. 2002).

We postulated the existence of a solicitation pheromone based on evolutionary considerations from the resolution of parent–offspring conflict (Godfray 1991; Parker et al. 2002b) and mechanisms regulating demand and supply in animal families (Hussell 1988; Parker et al. 2002b; Kölliker 2003). Given that mothers do not allocate the provisioned food to individual offspring (Brown et al. 2003), the scope for intraclutch sibling rivalry directed at mothers (Mock & Parker 1997; Rodríguez-Gironés 1999) driving the evolution of nymph solicitation traits is probably small in *S. cinctus*. Selection on nymph solicitation traits arising from parent–offspring conflict would therefore mostly act between clutches (Trivers 1974), provided there is a cost of provisioning in terms of residual reproductive success (Trivers 1974). A recent experimental study suggested that provisioning may be costly in terms of female survival even under ad libitum food conditions in the laboratory (Agrawal et al., in press). There is thus the potential for interclutch conflict driving the evolution of offspring solicitation traits in this species.

Our prediction that cuticular extracts from nymphs reared under low food would induce more maternal provisioning than extracts from nymphs reared under high food was supported by the data. Thus, a solicitation pheromone signalling nutritional condition might be involved in the short-term regulation of maternal provisioning in *S. cinctus*. The short-term nature of the extract

Table 2. Effects of extract exposure on the incidence of nymph shelter leaving

Quantification period	C1 ($N=20$)	C2 ($N=39$)	EXT-H ($N=39$)	EXT-L ($N=39$)	χ^2_3	*P*
0–2 h*	0	0	6^a (+6)	1^b (+1)	4.31	0.038
2–4 h*	1 (+1)	1 (+1)	7^a (+1)	2^a (+1)	3.30	0.069
4–6 h*	0 (−1)	0 (−1)	10^a (+3)	2^b	6.79	0.009
6–8 h*	0	2 (+2)	10^a (+1, −1)	3^b (+1)	4.73	0.030
Total†	1^a	3^a	11^b	3^a	9.94	0.019

Shown are the number of clutches that were found outside the shelter after the four 2-h measurement periods. Both the additional number of clutches leaving the shelter and the number of clutches returning under the shelter during a specific 2-h period are given (+ number leaving) and (− number leaving), respectively. Different alphabetical superscripts indicate significant treatment differences. See Table 1 for a description of treatment categories.
*Full model with all treatments was not possible because of too few cases of shelter leaving in some groups. Shown instead are likelihood ratio χ^2 statistics for the comparison between the EXT-H and the EXT-L treatment.
†The total counts all cases of shelter leaving occurring anytime during the whole exposure experiment. A full model across all four treatment groups is shown and contrasts were used for testing differences among individual groups.

effects suggests that the critical cues involved may be volatile compounds.

A solicitation trait by definition is produced actively by progeny and has stimulating effects on maternal provisioning (e.g. Trivers 1974; Godfray 1995; Parker et al. 2002b; Kölliker 2005). The unexpected overall inhibiting effect of nymph extracts on maternal provisioning is therefore contrary to the expectation associated with a simple solicitation pheromone, and we cannot firmly conclude without further experiments that a solicitation pheromone is really at work. There are several possible hypotheses that may account for this apparent paradox. A second chemical cue with an inhibiting effect may influence maternal provisioning independently of the solicitation pheromone. Such a compound could be an alarm pheromone produced by the nymphs during the handling before extraction (see Nault & Phelan 1984 for a review of hemipteran alarm pheromones, and Krall et al. 1997 for adult *S. cinctus* repellent compounds), or metabolic by-products (Cassill & Tschinkel 1995) or waste (Wyatt 2003) excreted by the nymphs. Mothers exposed to an alarm pheromone would be expected to increase time spent guarding and protecting the nymphs, possibly at the expense of food provisioning.

If metabolic by-products or waste is responsible for the observed decrease in provisioning, the prediction for maternal behaviour is less straightforward. Mothers may respond by decreasing time with nymphs to forage more for themselves, or by also increasing time spent with nymphs for guarding if self-feeding is not urgent. Unfortunately, given the extensive variability among females in their time budgets (Table 1; M. Kölliker, personal observation), the subsample used for the analyses of maternal time budgets turned out to be too small (59 versus 138 clutches in the full sample) for a powerful behavioural analysis. The data were therefore inconclusive on how females behaviourally adjusted their time budgets to produce the variation in food provisioning between treatments. There was a hint that the initial latency until females left the hut (which may be considered a measure of guarding) may be consistent with a trade-off between provisioning and guarding that was potentially modulated by extract exposure.

A single condition-dependent, passively produced and provisioning-inhibiting chemical cue could also potentially explain our results. If the amount of such a compound is a direct function of recent food intake and digestive activity (e.g. metabolic waste), such a cue may provide a mother with reliable information (Godfray 1991) about her progeny's recent food intake. It is not clear, however, how a communication system that is based only on inhibiting progeny cues would be evolutionarily stable in the presence of potential parent–offspring conflict (Parker et al. 2002b). It is important to consider, however, that other nymph traits and behaviours (e.g. tactile cues) may be involved in nymph solicitation (A. F. Agrawal, E. D. Brodie III & M. Kölliker, personal observation). If these behaviours have stimulating effects on maternal provisioning, inhibiting chemical cues may, in combination with stimulating signals, reflect nymph demand dynamically as a multidimensional compound-solicitation trait containing multiple messages (Johnstone 1996).

The differential effect of the extract exposure treatment on the incidence of nymph shelter leaving (i.e. dispersal) behaviour suggests an interaction between the effect of extract exposure, nymph shelter leaving and maternal provisioning. Because in our experiments both test mothers and test nymphs were exposed to the extracts and were allowed to interact with each other, the causal relationship among these effects is difficult to infer without further experiments. There are essentially four possibilities. First, the two effects may have been caused independently by the extracts to which both mothers and nymphs were exposed (i.e. independent cues for provisioning and shelter leaving). Second, nymph shelter leaving may have been indirectly induced by poor maternal provisioning, if low maternal provisioning induces nymphs, following a 'best-of-a-bad-job' strategy, to disperse. The high incidence of nymph dispersal in the EXT-H treatment (the treatment with lowest maternal provisioning), as well as the lack of provisioning by the mothers during the first 2 h of the exposure experiments of the seven dispersing clutches (M. Kölliker, unpublished data) is consistent with this hypothesis. The timescale for variation in provisioning to induce differential nymph dispersal seems rather short, however. Third, cuticular extracts may have induced shelter leaving by the nymphs directly (e.g. through an aggregation pheromone; Wyatt 2003), which may in turn have led to a decrease in maternal provisioning. Again, the timescale for such an indirect effect seems rather short. Furthermore, while exposure effects on maternal provisioning vanished after 2 h, they remained for nymph dispersal over the whole 8 h of the experiment. This result suggests that exposure effects on maternal provisioning and nymph dispersal are only loosely coupled behaviourally. Finally, if ontogenetic changes in the profile of the chemical cues occur, the difference in age between the nymphs used for extraction (second instar) and the test nymphs (first instar) might have lead to an artificial qualitative or quantitative mismatch in cues resulting in reduced maternal provisioning and enhanced nymph dispersal. However, such differences in age among nymphs within a family fit well within the range observed in the wild, where clutches consisting of different nymphal instars are readily observed (probably arising through clutch joining, or potentially the cohabitation of nymphs from successive clutches; E. D. Brodie, III, personal observation).

In summary, we have shown experimentally that chemical cues on the cuticle of *S. cinctus* nymphs are involved in the short-term regulation of maternal food provisioning and nymph dispersal behaviour. This is the first direct experimental demonstration that condition-dependent chemical cues by offspring affect parental food provisioning in a subsocial insect species. The unanticipated complexities of effects involved, including the potential for various combinations of provisioning-releasing solicitation pheromones and provisioning-inhibiting cues, as well as pheromones coordinating nymph dispersal behaviour (e.g. aggregation pheromones), emphasize the need for further experiments and the chemical identification of the compounds involved.

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