ORIGINAL ARTICLE

Aneil F. Agrawal · Jeremy M. Brown ·
Edmund D. Brodie

On the social structure of offspring rearing in the burrower bug, *Sehirus cinctus* (Hemiptera: Cydnidae)

Abstract Maternal-offspring interactions are important in a variety of animals. Understanding the evolution of these interactions requires that we also study the broader social context in which they occur. To date, behavioral studies on burrower bugs, *Sehirus cinctus*, have focused exclusively on interactions between mothers and offspring. Here we ask whether these interactions occur in a social context that extends beyond the family unit of a mother and her own genetic offspring. Such social structure can arise from behaviors that occur before eggs are laid, or from actions of individuals that occur posthatching. We present field data showing that lay sites of mothers are spatially aggregated on a scale that would lead to behavioral interactions among families. Microsatellite markers suggest neighboring mothers are unrelated. Laboratory experiments do not support the hypothesis that spatial aggregation results from a direct attraction of females to one another. Other laboratory studies reported here indicate that, after hatching, unrelated clutches sometimes join together to form multifamily groups. Experiments reveal that mothers are not necessary for these joining events to occur. In sum, these data suggest that both mothers and offspring play active, but different, roles in generating the social environment in which offspring rearing occurs.

Keywords Communal breeding · Social structure · Burrower bugs · *Sehirus cinctus* · Maternal care

Communicated by N. Wedell

A. F. Agrawal (✉) · J. M. Brown · E. D. Brodie III
Department of Biology and Center for the Integrative Study
of Animal Behavior,
Indiana University,
Bloomington, IN 47405, USA
e-mail: aagrawal@bio.indiana.edu
Tel.: +1-604-8225401
Fax: +1-604-8222416

Present address:
A. F. Agrawal, Department of Zoology,
University of Toronto,
Toronto, Canada

Introduction

In a variety of animal systems, parents care for their offspring. These parental responsibilities can include feeding, cleaning, and protecting offspring (Clutton-Brock 1991). In a subset of systems exhibiting parental care, there is an added dimension of social complexity. When multiple females are rearing offspring in the same time and place, the potential network of social interactions is greatly expanded. Mothers may interact with their own offspring, as well as with other mothers and other offspring. Such behaviors are observed in many taxa including spiders (Avilés 1997), Hymenoptera (Eickwort 1981), webspinners (Order Embiidina) (Edgerly 1997), salamanders (Harris et al. 1995), birds (Stacey and Koenig 1990), and mammals (Solomon and French 1997a). In some cases, mothers simply share overlapping nest sites, but in others cooperation is more extensive, with mothers feeding and protecting each other's offspring. We use the term "communal nesting/breeding" to include any case where the potential exists for offspring to have extensive interactions with non-siblings and/or adults other than their own genetic parents; cooperation is not implied (see Brown 1987; Solomon and French 1997b for a discussion of this and other related terms).

Foremost among the potential benefits of communal breeding are inclusive fitness gains via kin selection (Hamilton 1964). Indeed, many cooperative breeding groups consist of relatives (e.g., Reeve et al. 1990; Woolfenden and Fitzpatrick 1990; Crozier and Pamilo 1996). However, kin selection is no longer regarded as a universal explanation for cooperative breeding because relatedness alone is not a powerful predictor of whether a species breeds cooperatively and, in some systems showing cooperation, non-relatives participate as much as relatives (Clutton-Brock 2002). Nonetheless, kin selection is an important phenomenon that must be considered among potential explanations of the evolution of social behaviors.

A number of other potential benefits of communal breeding have been suggested that are independent of

relatedness among interactants. Communal breeders may be better able to defend their offspring from predators (e.g., Austad and Rabenold 1985; Mappes et al. 1995) or aggressive conspecifics (Packer et al. 1990; Manning et al. 1995). Communal breeders also may be able to provide offspring with more food or feed them at more regular time intervals (Emlen 1990; Wenzel and Pickering 1991). If a mother dies, her offspring can be raised by the other mothers in her group (Strassmann 1981). Such direct benefits can occur even in the absence of kin selection and may be the driving force in the evolution of communal breeding in some systems.

Regardless of why it occurs, communal breeding changes the social context for both mothers and offspring. This expanded social context is important for two reasons. First, this social environment may generate selection for new cooperative or competitive behaviors. To the extent that communal breeding groups consist of relatives, kin selection will be involved in shaping these selection pressures (Hamilton 1964; West et al. 2002). Second, the evolution of new behaviors specific to this extended social context can change existing maternal and/or offspring behaviors if new and old behaviors are genetically correlated (Arnold 1994).

In situations where obvious communal structures are not present, evidence of communal nesting can be sought by showing that sites of reproduction (e.g., lay sites) are spatially aggregated. Such evidence alone is not definitive proof of communal nesting for two reasons. First, families might operate independently of one another even if they are aggregated, particularly if the scale of individual interactions is small relative to the scale of aggregation, or if families actively avoid one another. Aggregation must occur on a scale that is biologically relevant to the species of interest and must result in interactions among families. Second, lay site may not equate with the rearing site if families move after hatching/birth (e.g., Williams 1974; Munro and Bédard 1977; Levy and Bernadsky 1991). The distribution of rearing sites could be more or less aggregated than the distribution of lay sites.

However, these two distributions are not completely independent. Rearing-site distribution is much more likely to be aggregated if the lay-site distribution is aggregated than if it is over-dispersed. For example, in the colony of white pelicans studied by Evans (1984), the average inter-nest distance was less than 1 m, making it easier for offspring from different nests to interact than if nests were more widely separated. Within the constraints of available habitat, mothers typically control the distribution of lay sites. Through their choices of lay sites, mothers can promote or avoid the aggregation of rearing sites. If mothers also control the relocation of families after birth/hatching, then they also determine the aggregation of rearing sites and the potential for communal breeding. However, offspring might play an active role in the movement of the family after birth/hatching (e.g., Williams 1974; Munro and Bédard 1977). If so, communal breeding might be due, at least in part, to the ac-

tions of offspring rather than solely the result of parental decisions.

In the burrower bug, *Sehirus cinctus*, mothers guard egg clutches and provision juvenile offspring with mint nutlets. Previous studies indicate this is a bidirectional interaction in which offspring elicit provisioning, though the mechanism is unknown. Further, this work suggests that mothers are genetically variable in their tendency to provision, and offspring are genetically variable in their ability to elicit provisioning (Agrawal et al. 2001). To date, maternal-offspring interactions in this system have been studied in isolation (i.e., a single mother and her offspring). Although some mothers likely raise their offspring in social isolation, field and laboratory observations have hinted that other mothers may experience a more complicated social environment (Kight 1995; A.F. Agrawal and E.D. Brodie III, personal observations). These observations warrant a more rigorous investigation of the social breeding context in this system. Our primary goal here was to determine whether families exist in relative isolation or are likely to interact with one another during offspring development. Secondarily, if families do interact, we sought to determine whether this was due to the actions of parents or offspring (i.e., which generation determines social structure).

Here we report data from field and laboratory studies that suggest that maternal-offspring interactions sometimes occur in larger social groups. We sampled field distributions of lay sites to determine if they were aggregated or distributed randomly. To determine the potential importance of kin selection in interactions among neighbors, we investigated whether neighboring females were related. We used experimental laboratory studies to investigate whether females actively choose to aggregate their lay sites based on the presence of other females, and to determine whether and how families move from their initial lay site after hatching and become aggregated. We report evidence that some families move to join other families. The last experiment reported here examines the proximate reasons for why joining occurs and whether offspring play an active role in determining their social situation.

Methods

The study organism

The burrower bug (*Sehirus cinctus cinctus*, Hemiptera: Cydnidae) is found from the northeastern United States south to Mexico (Froeschner 1960). As in much of their range, burrower bugs in southern Indiana can be found in the spring feeding on the nutlets of the weedy mints *Lamium purpureum* and *L. amplexicaule*. Burrower bugs overwinter as adults and emerge in the spring to feed and reproduce. Although little is known about this species and some reproduction might occur throughout the spring and summer, the bulk of reproduction seems to occur in late April–early May as the mint plants set seed and begin to senesce (Sites and McPherson 1982; A.F. Agrawal, personal observations). At this time, there is a noticeable shift in activity over a period of approximately 1 week from the majority of the bugs feeding on plants, to females remaining on the ground guarding eggs (A.F. Agrawal, personal

observations). Females lay clutches of eggs on the ground, often under cover objects or in small depressions, and guard these eggs for approximately 10 days until they hatch (Sites and McPherson 1982; Kight 1995). After hatching, mothers gather mint nutlets that they provision to their offspring (Sites and McPherson 1982). In the laboratory, mothers provision offspring through the first two juvenile instars (Agrawal et al. 2001). During this time, offspring are gregarious.

Spatial distribution of female lay sites in the field

The field site used for this study was a 50 m×20 m patch at the edge of an agricultural field in Monroe County, Indiana, United States, which was covered predominantly with *L. purpureum*. Throughout the midwestern United States, *L. purpureum* and *L. amplexicaule* commonly cover large portions of agricultural fields before they are plowed in the spring. Burrower bugs can often be found in such fields. Though such sites may not be representative of typical burrower-bug habitats over most of their evolutionary history, agricultural fields likely represent an important habitat over the past century or more.

Field data were collected between 27 and 30 April 2000, during the period after *L. purpureum* had begun to senesce and when females were rarely found on the plants but were commonly found on the ground with eggs. Within the large patch, potential locations for study were chosen haphazardly in areas covered with the mint. Each location was initially scanned for the presence of females with eggs. If an initial scan revealed the presence of at least two females with eggs, systematic sampling proceeded. In such locations, a quadrat of 50.5 cm×56.5 cm was investigated. In each quadrat, all of the plants were clipped to the ground and all cover carefully removed. The top layer of soil was systematically excavated to the depth where the ground was too hard to dig with a small spatula (0.5–2 cm). The location of each clutch of eggs (i.e., lay site) was recorded on an [x, y] grid relative to the quadrat perimeter. Typically, an egg clutch was being guarded by a female (the presumed mother), but in some cases egg clutches were found without an associated female, likely because the female had been accidentally frightened away during the survey process. When females could be accurately assigned to egg clutches, they were collected for molecular analysis (see below). In total, five quadrats were investigated; each quadrat was at least 2 m away from any other quadrat. The areas investigated (both within and among quadrats) were relatively homogeneous with respect to plant cover and quality.

There are a variety of ways to measure spatial distribution (Diggle 1983). For this system, nearest-neighbor distance is both biologically relevant and easy to interpret. For the *i*th quadrat, the mean nearest-neighbor distance (D_i) was calculated by averaging the nearest-neighbor distance for each lay site (d_{ij}, $j=1,...,n_i$; where n_i is the number of lay sites found within quadrat *i*). For each quadrat, Monte Carlo simulations were performed to test whether lay sites were randomly distributed (Diggle 1983). In a simulation for quadrat *i*, n_i lay sites were randomly assigned to positions within a 50.5 cm×56.5 cm area. Mean nearest-neighbor distance was calculated for the simulation. One thousand such simulations were performed for each quadrat to generate a distribution of mean nearest-neighbor distances expected under the null hypothesis of complete spatial randomness. The observed mean nearest-neighbor distance for each quadrat was then compared to the corresponding null distribution.

Using molecular markers to test for relatedness among neighboring mothers

We measured the relatedness among females collected in the field study using microsatellite markers. Genomic DNA extractions were performed by combining one individual, two 3-mm tungsten-carbide beads (Qiagen), and 300 μl cell lysis solution (Gentra) in a 1.5-ml microfuge tube. Tubes were shaken by hand for 1 min, or until individuals were sufficiently ground. Extractions were then per-

formed using a Puregene extraction kit (Gentra). Extracts were usually diluted by 1/5 for use in PCR amplification of microsatellites.

Primers for three variable microsatellite loci (A6, A7, and A11) in *S. cinctus* were obtained from a previous study of paternity in burrower bugs (Brown et al. 2003), while a fourth locus (A2) was designed and characterized according to identical methods. The forward primer sequence for locus A2 is 5′ AGCTTGACTGGC-GAAGGATA 3′, while the reverse primer sequence is 5′ TT-GATCAGACTCCCAACGTC 3′. Primer oligonucleotides were synthesized by Invitrogen. The following thermal profile was used for PCR amplification of all microsatellite loci: 8 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min 45 s at 72°C, with a final extension step of 10 min at 72°C. Total reaction volume was 25 μl and included 0.5 μl of 1/5 diluted DNA extract, 0.5 U *Taq* polymerase (Promega), 3.5 mM MgCl$_2$, 1X PCR Buffer (Promega), 0.2 mM dNTPs (NEB), and 0.25 μM each of forward and reverse primers. Forward primers were either Hex- or Fam-labeled, run against an R500 Gensize standard (Genpak), and visualized using Genescan capabilities on an ABI 3700 sequencer (Applied Biosystems). Genotyping was performed using Genotyper v3.7 (Applied Biosystems).

Pairwise relatedness was calculated using the method described by Lynch and Ritland (1999). To determine if individuals that nest close together are more closely related than individuals that nest apart, we asked if pairs of females that nested less than 2, 5, 10, or 20 cm apart were more closely related than the average relatedness between individuals in different quadrats (>200 cm apart). To test whether our observed differences were statistically significant, we performed a permutation test (Good 1994). In each of 1,000 permutations, observed genotypes were randomly re-assigned to observed positions across the quadrats and the difference in relatedness of near and distant pairs was calculated. These permutation results provided a null distribution against which we could test our observed results.

Experiment 1—proximate reasons for spatial aggregation

As reported below, lay sites in the field were aggregated. There was no obvious environmental heterogeneity or physical limitation (e.g., stones or logs that would prevent nesting in some spots) that was associated with the distribution of lay sites within quadrats. These aggregations could occur because nesting females are directly attracted to each other or because they tend to be attracted to similar microhabitats (i.e., nesting females attend to environmental heterogeneity that is not readily detected by humans). We used experimental arenas to test whether females were directly attracted to each other.

Each arena consisted of a large (150×15 mm) petri dish containing a small (35 mm diameter) dish in the center. This central dish was filled with water-saturated sand and covered with a lid containing small holes and served only as a humidity reservoir. Bugs could not get into this dish. Four other small plastic dishes were spaced evenly around the perimeter of the arena (Fig. 1). Each of these smaller dishes contained moistened sand and a small plastic cover object. Bugs could readily climb into these dishes. These four small dishes were intended as good lay sites and are referred to as laying dishes. We attempted to minimize any variation among laying dishes within an arena. These laying dishes were numbered 1–4 in a clockwise direction with laying dish 1 being assigned at random. No additional substrate was provided elsewhere in the arena.

In experiment 1A, we tested whether a gravid female was more likely to lay in a laying dish already containing a nesting female than in an unoccupied dish. Gravid females (easily identified by distended abdomens) were housed individually in standard (100×15 mm) petri dishes containing moistened sand and checked daily for egg laying. On the day egg laying was observed, a female with her eggs was carefully transferred into laying dish 1 of a new experimental arena. This female was referred to as the "seed" female. Seed females always stayed in the dish to which
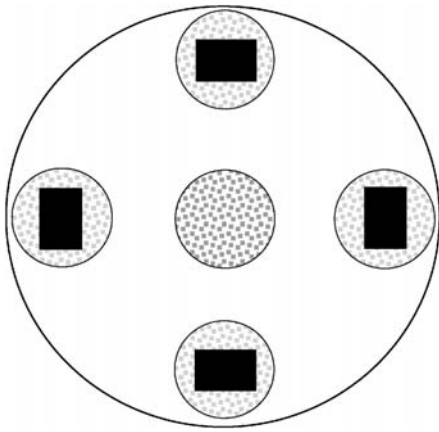
Fig. 1 Arena for experiment 1. The *central mottled grey circle* represents the humidity reservoir. The other *mottled grey circles* represent laying dishes with dampened sand substrate. Darkly *shaded rectangles* represent plastic cover objects. See text for further explanation.
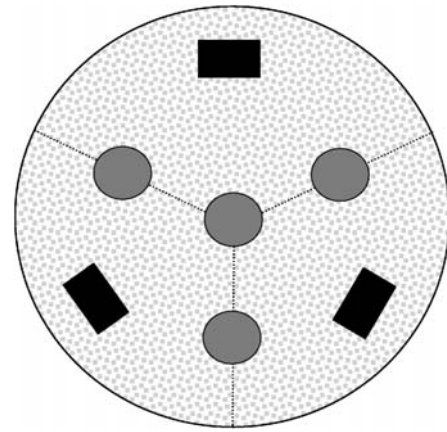

Fig. 2 Arena for experiment 2. *Solid shaded circles* represent food dishes. Darkly *shaded rectangles* represent plastic cover objects. *Mottled grey background* represents dampened sand substrate. *Dashed lines* represent imaginary boundaries separating the three zones. See text for further explanation.

they were transferred. A single gravid female (that had not yet laid eggs) was chosen at random and added to the arena. This second female was referred to as the "response" female. Arenas were checked daily to see if and where the response female had laid. Experiment 1A included 34 replicates using unique females.

Because we assigned the seed female to laying dish 1, rather than letting her choose a lay site, there should be nothing special about this laying dish per se. If response females laid in (or near) laying dish 1 more often than expected by chance (25%), then we would interpret this as strong evidence that response females were attracted to this dish because of the presence of the seed female.

Experiment 1B was set up in a similar fashion, except that two gravid females were placed in the arena simultaneously and allowed to choose their own lay sites. Though we attempted to construct all laying dishes in the same way, it is possible that variation in laying-dish quality could exist and contribute to any joint nesting (i.e., both females might choose to lay in the same dish because it was the best lay site). Thus, joint nesting in experiment 1B might occur either because of microhabitat choice or social aggregation. However, a failure to observe joint nesting in both 1A and 1B would provide evidence that any lack of joint nesting in 1A did not result merely from excessive experimental manipulation and differences in timing of laying between the two females. Thirty-four replicates of experiment 1B were conducted, but one replicate was excluded from analysis because both mothers in this replicate behaved uncharacteristically and failed to tend eggs.

Binomial tests were used to determine if the frequency of joint nesting was statistically different than expected under a hypothesis in which the laying-site decisions of females were not affected by other females.

Experiment 2—the movement of families after hatching

The original intent of experiment 2 was to test whether mothers who nest communally have a competitive advantage over those that nest independently. As described below, mothers with eggs were placed either alone or in pairs under different cover objects within the same arena so that they would be in direct competition for the available resources. The experimental design did not fulfill its original purpose because of the unanticipated post-hatching movement of families that had not been observed in single-family experiments. The results are presented here because they demonstrate that families frequently move after hatching to join other families. Further, the results of this experiment, in combination

with experiment 3 (below), hint at proximate explanations for why joining occurs.

In experiment 2, each arena consisted of a large disposable petri dish (150 mm×15 mm) with a dampened sand substrate. Three plastic cover objects were placed equidistant (~90 mm) from one another (Fig. 2). Food dishes (beer-bottle caps) were placed in between each cover object and at the center. These food dishes also served as boundaries dividing the experimental arena into three sectors for purposes of data collection. Food dishes each contained ten *L. purpureum* nutlets and were replenished daily as needed. Under each of two cover objects, a single mother with her egg clutch was placed. Two mothers, each with her own egg clutch, were placed under the third cover object. For a given replicate, all mothers had laid their egg clutches on the same day, each in its own husbandry chamber (60 mm diameter). Two to seven days after egg laying, each female was carefully marked with a unique spot of paint on the pronotum for identification, and delicately transferred to her randomly assigned location in the experimental arena. All females for a given replicate were processed on the same day.

Twenty-four replicates were set up and checked daily for hatches. Data collection began when the first clutch hatched (approximately 10 days after laying) and continued for the next 4 days (i.e., before any offspring reached the second instar). In 22 replicates, all 4 clutches hatched within 2 days of the first hatch; of those, 20 hatched within 1 day of each other. Given the short duration of the experiment, the results for the remaining three replicates are not presented because the hatches were too asynchronous. Their inclusion does not change the overall pattern.

During each day of data collection, the location of each mother was recorded. Because each arena contained 4 large clutches (~90±30 offspring), and we are unable to mark offspring, it was not possible to track individual offspring. However, when there were no or very few offspring in a sector, it could be safely assumed that the offspring originally assigned to that sector had moved elsewhere. Because first-instar offspring tend to move in fairly cohesive groups, it was possible to identify the sector to which they had moved by visually assessing the number of offspring in the other sectors. Further, there is among-family variation in offspring size and color and this variation helps in assessing whether the offspring in a sector originate from one, two, or three families. For these reasons, we are quite confident in our ability to infer the movement of offspring.

As mentioned above, we are unable to use this experiment for its originally intended purpose so we did not perform a statistical test. We simply present the patterns observed with respect to
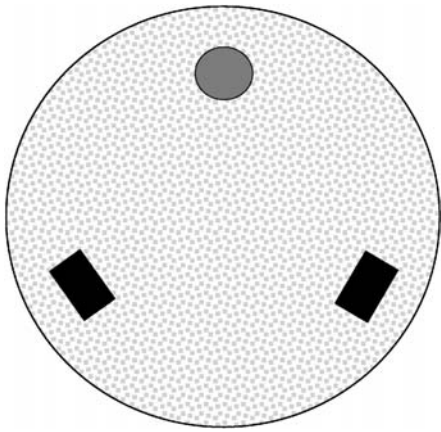
Fig. 3 Arena for experiment 3. The *solid shaded circle* represents a food dish. Darkly *shaded rectangles* represent plastic cover objects. *Mottled grey background* represents dampened sand substrate. See text for further explanation.

joining events that are relevant to understanding why these events occur.

Experiment 3—factors affecting the probability of a joining event

The results of experiment 2 indicated that post-hatching joining events occur but they did not tell us how or why. For example, do mothers actively lead their offspring to join other clutches or do offspring simply leave their cover objects if they are not being adequately provisioned? To pursue these issues, we conducted an experiment in which we examined the importance of (i) mothers, and (ii) food availability, on the tendency of offspring to join with other families.

Each experimental arena consisted of a large disposable petri dish (150 mm×15 mm) with a sand substrate. Two plastic cover objects and one food dish were placed approximately equidistant (~90 mm) from one another (Fig. 3). The food dish contained 50 nutlets and was replaced daily. Prior to the start of this experiment, the mothers of the offspring used in this experiment were housed individually in small petri dishes (100 mm×15 mm). Mothers laid eggs in these dishes and were able to guard these eggs until they hatched. The day after hatching, mother and offspring were chilled on ice to allow offspring to be counted and transferred. Under each of the two cover objects within an experimental arena, we placed 60 (1 day old) offspring and half of the empty egg cases of the clutch. All 60 of the offspring placed under a cover object came from a single clutch and each clutch was used to stock a single cover object only. The two clutches used within a replicate hatched on the same day.

Experiment 3 was set up as a 2×3 factorial design varying food resources and presence of mothers. Food was either "supplied continuously" (SC) or "supplied once" (SO). In the SC treatment, three nutlets were supplied to each cover object daily. In the SO treatment, three nutlets were supplied to each cover object only once, on the day offspring were transferred to the experimental arena. Each replicate was also assigned to one of three mother treatments: "zero mothers" (0 M), in which no mothers were present in the experimental arena; "one mother" (1 M), in which the mother of one of the two clutches was also transferred with her clutch to the appropriate cover object but the remaining mother was not placed in the arena; or "two mothers" (2 M) in which each of the two mothers was transferred to the appropriate cover object with their respective clutches. When present, mothers were able to carry nutlets from the food dish to offspring (i.e., provisioning). Combining the "supply" and "mother" treatments resulted in 6

unique factorial treatments; 11–15 replicates were completed for each cell of the factorial design.

Data were collected once daily for 5 days or until joining of clutches occurred. Each day, the locations of mothers and clutches were noted. For replicates in the SO treatment, we also attempted to note whether a mother had done any additional provisioning to offspring. Joining was judged to have occurred when the majority of offspring were found in a single cluster. A total of 78 replicates provided clear results. In four other replicates, results were ambiguous as to whether a joining event had occurred (e.g., only half of one clutch had joined the other clutch); these four replicates were not included in the analysis.

To determine the effects of "supply" and "mothers" on the probability of a joining event, we fit a logistic model using the GENMOD procedure in SAS (1999), with supply as a class variable and number of mothers as a continuous variable. Likelihood ratio tests were used to test for significance of effects.

Results

Distribution of lay sites in the field

Females with eggs were often found on the soil beneath litter or in depressions or small caverns in the soil. Some bugs with eggs were found in relatively exposed positions. Though the surface soil was carefully excavated, no burrower bugs were found completely buried in the soil without some obvious opening on the surface. No hatched eggs or juvenile offspring were found. The distribution of lay sites for each quadrat is shown in Fig. 4. The number of lay sites found in the five quadrats was quite variable (Table 1). Nonetheless, in each quadrat, the mean nearest-neighbor distance was small (less than 5.5 cm for all quadrats) and less than expected under complete spatial randomness ($P<0.01$ for each quadrat, Table 1), indicating that lay sites were aggregated.

Relatedness of neighbors

We found no evidence that neighboring females were closely related. Point estimates of average relatedness were near zero for all distances considered (Table 2). The difference in relatedness between pairs of close versus distant neighbors always fell below the 95th percentile of the null distribution generated by permutation (i.e., $P>0.05$ for all distances).

Experiment 1—proximate reasons for spatial aggregation

In experiment 1A, 29 out of the 34 response females laid within 1 of the 4 laying dishes. The remaining five females could all be unambiguously assigned as laying closer to one of the dishes than to any of the other three. All response females laid within 3 days of their respective seed females; 33 out of 34 laid within 2 days. Only 5 out of 34 females (~15%) laid in laying dish 1 (i.e., in the same dish as the seed female). The frequency of nesting in (or near) the same laying dish as the seed female is not

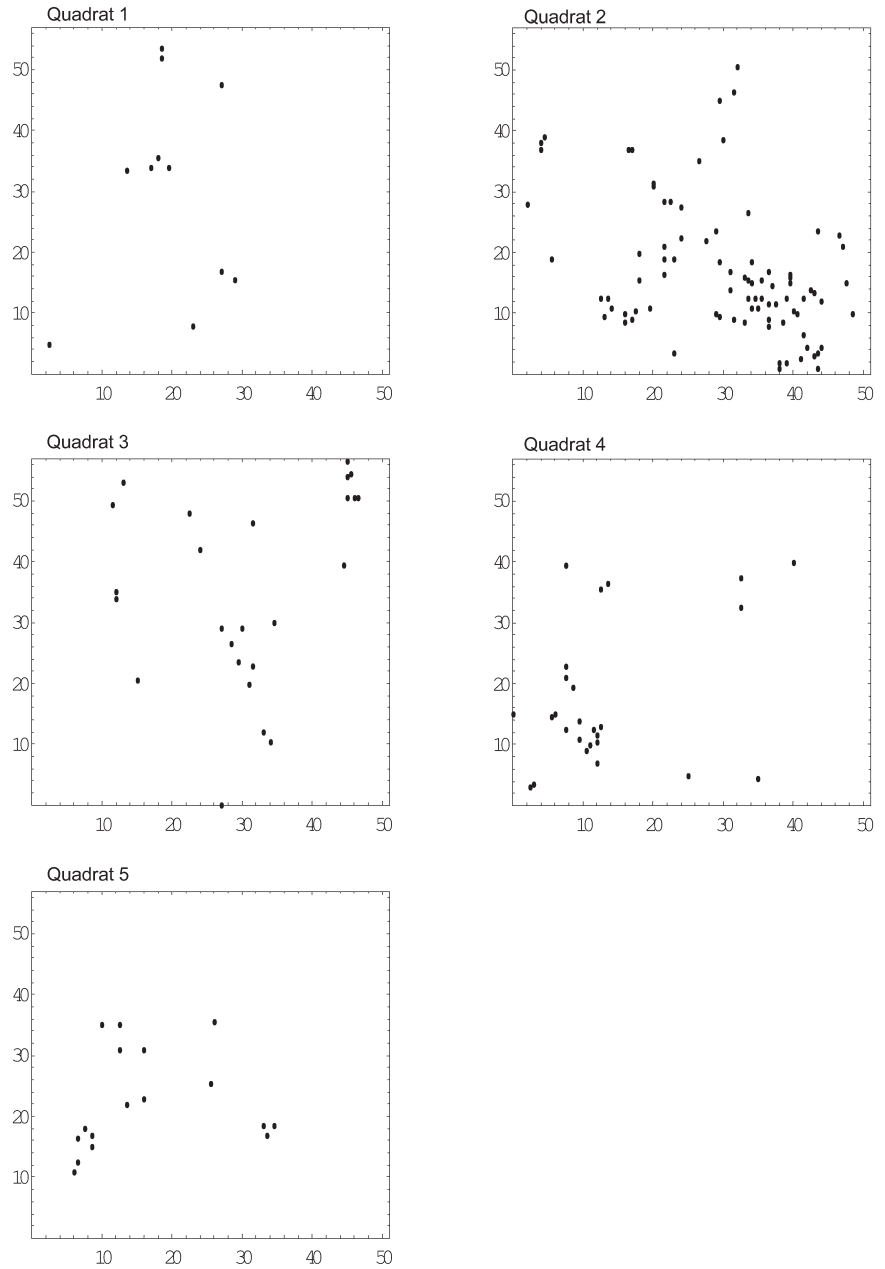**Fig. 4** Distribution of lay sites in the field. The dimensions of each quadrat were 50.5 cm×56.5 cm.

Table 1 The aggregation of lay sites in the field. The third column gives the observed mean nearest-neighbor distance. The fourth column gives the average mean nearest-neighbor distance observed in 1,000 simulations assuming complete spatial randomness. The last column shows the frequency (*P*) of simulated mean nearest-neighbor distances that were less than the observed mean nearest-neighbor distance (*D*) (* indicates that no simulations produced mean nearest-neighbor distances as small or smaller than the observed value)

Quadrat	Number of lay sites	Mean nearest-neighbor distance [*D*] (cm)	Expected mean nearest-neighbor distance (cm)	*P<D*
1	11	5.20	9.4	0.006
2	86	1.95	3.0	<0.001*
3	25	3.91	5.8	0.001
4	26	2.96	5.7	<0.001*
5	17	3.04	7.3	<0.001*

Table 2 Relatedness of close neighbors. The data presented in this table combine information from all plots. The second column shows the number of pairs for which genotype information was available to estimate relatedness. The third column gives the average relatedness of these pairs. The fourth column shows the difference in average relatedness between near neighbors and mothers in different quadrats, r'. The final column gives the frequency of permutations that resulted in r' values greater than the observed value

Type of neighbors	Number of pairs	Relatedness	Relatedness of close vs distant neighbors [r']	$P > r'$
Within 2 cm	52	0.06	0.07	0.07
Within 5 cm	268	0.01	0.02	0.11
Within 10 cm	730	0.00	0.01	0.15
Within 20 cm	1720	0.00	0.01	0.96
Among quadrats	7526	−0.01	–	–

Table 3 Effect of nutlet supply and the presence of mothers on joining

Supply treatment	Number of mothers	Number of replicates	Number of replicates where joining occurred (%)
Supply continuously (SC)	No mothers (0 M)	11	2 (18%)
	One mother (1 M)	12	2 (17%)
	Two mothers (2 M)	13	5 (38%)
Supply once (SO)	No mothers (0 M)	14	14 (100%)
	One mother (1 M)	13	10 (77%)
	Two mothers (2 M)	15	8 (53%)

significantly different than expected by chance (two-tailed binomial test $P < 0.233$).

In experiment 1B, 57 out of 66 mothers laid within 1 of the 4 laying dishes. Eight of the remaining nine mothers could be unambiguously described as laying closer to one dish than to any of the others. The last mother's laying position was ambiguous and her replicate is excluded from the results below. In 9 out of the 32 unambiguous replicates (28%), both females laid in (or in close proximity to) the same laying dish. The observed frequency is not significantly greater than that expected under a hypothesis of no mutual attraction (one-tailed binomial test $P < 0.406$).

Experiment 2—the movement of families after hatching

In 6 out of 22 (27%) replicates, there was no evidence of joining. In six other replicates (27%), all (or most) of the offspring were missing from one of the two single-clutch sectors by the end of the experiment, indicating that they had moved to another sector and had joined another group of offspring. In the remaining ten replicates (45%), all (or most) offspring were in the two-clutch sector by the end of the experiment, indicating that both of the independent clutches had joined the original pair of clutches. Considering all the replicates together, there was evidence for joining in 16 out of 22 replicates (73%). In general, all offspring within a sector formed a somewhat cohesive group; usually distinct offspring groups were not obvious within a sector. There was no indication that clutches would join temporarily and then split up again. The majority of offspring never vacated the two-clutch sector in any of the replicates.

Experiment 3—factors affecting the probability of a joining event

Joining events occurred in 41 out of 78 total replicates (53%) but were not distributed evenly across the treatments (Table 3). The logistic model including effects of supply, number of mothers, and their interaction (i.e., the full model) was significantly better than the null model of no effects (likelihood ratio statistics: $\chi^2 = 32.59$; $df = 3$; $P < 0.0001$). Likelihood ratio tests of the model effects showed that the effect of supply was significant ($\chi^2 = 21.33$; $df = 1$; $P < 0.0001$). As shown in Table 3, joining occurred much more frequently in the "supplied once" (SO) treatment (76%) than in the "supplied continuously" (SC) treatment (25%). There was no main effect of number of mothers on frequency of joining ($\chi^2 = 1.86$; $df = 1$; $P = 0.17$). However, there was a significant interaction between supply and number of mothers ($\chi^2 = 9.38$; $df = 1$; $P = 0.006$). This interaction may occur because mothers, when present, can provision offspring, thus changing the importance of the SO versus SC treatments.

Discussion

The number and type of possible social interactions for any species depends on the spatial distribution of individuals. It is important to quantify such distributions and to understand how they arise to determine what potential they have to influence the evolution of social behaviors. We have begun to study spatial distribution in burrower bugs during a period that is of particular importance with respect to social evolution—the initial period of maternal care.

The distribution of lay sites

Our field data show that lay sites were aggregated (Fig. 4); average nearest-neighbor distance was less than expected under complete spatial randomness (Table 1). There are at least two (non-exclusive) reasons for why this pattern occurs. The first possibility is that nonrandom distributions may occur because females prefer to lay close to other nesting mothers. However, experiment 1 failed to provide any support for this hypothesis; females did not preferentially choose to nest with another female in a laboratory setting.

The second possible explanation for the aggregation of natural lay sites is that females are attracted to the same microhabitats because of their desirable physical characteristics. Although the physical attributes of different locations within quadrats were not systematically assessed, there were no visually obvious differences (e.g., soil type, density of *L. purpureum*, type or abundance of litter) between areas where lay sites were aggregated and areas that contained few or no burrower bugs. Of course, bugs may be sensitive to variation that is not easily observed by humans. Because experiment 1 did not support the idea that females are directly attracted to other females, we propose that variation in physical properties among lay sites is the more important explanation for the observed aggregations. Direct experimental evidence of this conjecture is needed.

The aggregation of lay sites increases the potential for interactions between families. The evolution of such interactions will be molded by kin selection (Hamilton 1964) if neighbors are related to one another. However, we can exclude kin selection as being an important evolutionary force here as there is no evidence that females that laid close to one another were closely related. Close pairs were not significantly more related than distant pairs. Moreover, point estimates of relatedness between close pairs were very low (r=0.00–0.06). These results contrast with recent data from another subsocial Hemipteran, the lace bug *Gargaphia solani*, in which individuals that contribute to mixed egg broods tend to be related (Loeb 2003).

The post-hatching social environment

Regardless of why it occurs, the aggregation of lay sites could have consequences for the social environment of reproduction. After hatching, females leave their offspring to find mint nutlets to provision their clutch. In the laboratory, we have frequently observed females travel >25 cm to collect mint nutlets for their offspring (A.F. Agrawal, N. Combs, and E.D. Brodie III, unpublished data). Average mean nearest-neighbor distance in the field was <6 cm. Thus, females could either compete with one another for mint nutlets or even steal mint nutlets collected by other mothers (e.g., Filippi et al. 2002). The important point is that lay sites are aggregated on a scale that is biologically relevant with respect to the behavior of the insects.

Even though we had reasons to believe that mothers would encounter other families, we had not previously considered that offspring from different families would encounter each other. In single-family studies (Agrawal et al. 2001), the first-instar offspring typically remain close to their hatch sites in tight clusters that are less than 2 cm in diameter. Therefore, it was conceivable that clutches laid more than a few centimeters apart would remain independent. We had not anticipated the post-hatching movement of first-instar offspring. In contrast to our expectations based on single-family studies, experiment 2 showed that clutches separated by ~9 cm often merge into multifamily groups.

Experiment 3 was designed to examine factors that influence the probability of a joining event. Our original hypothesis was that a mother actively leads her clutch to join another clutch, predicting that joining would be least common in the 0 M treatment and most common in the 2 M treatment. However, our data did not support this hypothesis. Instead, we found that nutlet availability was the most important factor in determining whether a joining event occurred: when insufficient food was supplied to offspring (the SO treatment), joining occurred frequently. Offspring were much more likely to join another clutch than to simply move to the well-stocked food dish, suggesting that offspring were attracted, in the proximate sense, to other clutches of conspecifics rather than the food source directly.

Though the exclusion of mothers and/or the provisioning of food directly to offspring are useful experimental manipulations, these conditions are unnatural. Experiment 2, in which all clutches had mothers and offspring were not supplied with food directly by experimenters, represented the most realistic scenario. Joining occurred frequently in experiment 2, suggesting that it may occur frequently in the field. Due to the semi-fossorial nature of these insects, it is impossible to directly observe joining under natural conditions. Molecular markers will be required to determine the frequency of joining in the field. However, groups of mixed-age offspring are commonly found, providing indirect evidence that some joining of unrelated individuals occurs in nature.

Comparison with social structures of other taxa

In many examples of communal nesting, social structure is determined by adults; adults form or join groups, packs, colonies, etc. and offspring are born into them (e.g., Wilson 1971; Stacey and Koenig 1990; Solomon and French 1997a). However, if offspring are not completely altricial, then they may take a more active role in determining their social environment. In their discussion of waterfowl, Eadie et al. (1988) coined the term "post-hatching brood amalgamation" which "occurs when a female incubates and hatches her own young, but the

young mix with the brood of another female after hatching."

Of the situations covered by this term, our system seems most similar to some examples of crèching behavior. Munro and Bédard (1977) described brood mixing and crèche formation in the common eider, *Somateria mollissima*, as occurring in response to the mutual attraction of ducklings or in response to predation attempts. "Superbroody" mothers seemed to attract more ducklings. They viewed stable crèche formation as an accidental expansion of the family that occurs due to overcrowding. In white pelicans, *Pelecanus erythrorhynchos*, crèche formation occurs while their parents are away feeding and seems to result from the attraction of offspring to each other (Evans 1984). In the shelduck, *Tadorna tadorna*, ducklings sometimes form mixed broods despite the efforts of their parents to keep families separated (Williams 1974). In contrast, crèche formation in the Nubian ibex, *Capra ibex nubiana*, results from parents actively leading their offspring to the crèche (Levy and Bernadsky 1991).

Another important aspect of the ibex system is that mothers appear to feed only their own offspring and not unrelated members of the crèche. This feature is shared by some other systems with both crèche formation and maternal provisioning (e.g., Evans 1984; Velando 2001). In contrast, burrower-bug mothers would be unable to exclusively provision their own offspring because nutlets are delivered to the brood as a whole, not to individuals.

According to Filippi et al. (2001), there are only three confirmed examples of progressive provisioning within the hemipteran family Cydnidae: *Sehirus cinctus*, *Parastrachia japonensis*, and *Adomerus triguttulus*. Though little is known about the last (see Nakahira 1994), considerable effort has been spent on studying maternal care in *Parastrachia japonensis* under natural conditions (reviewed in Filippi et al. 2001). Mothers tend eggs and provision offspring with drupes of the host tree *Schoepfia jasminodora*, often dragging these drupes over distances 5–15 m. Although there has been no formal study of social structure in this system, there is anecdotal evidence for occasional joining in response to low food availability; offspring have been observed to leave poorly provisioned nests to join other nests (Tsukamoto and Tojo 1992).

Conclusion

In sum, we have found evidence that lay sites are aggregated and that neighboring mothers are not related. This initial aggregation of families is exacerbated by the post-hatching movement of offspring. Taken together, these observations indicate that mothers and offspring each play different but active roles in determining the social structure of this species. Joining seems to occur, in part, because offspring do not receive food at their original hatching site rather than because mothers actively lead their offspring to another clutch.

The data presented here help to build a more complete picture of the social context in which maternal-offspring interactions occur. These data document patterns and provide some insights into the proximate causes of these patterns. The evolutionary forces shaping these patterns are as yet unknown, though kin-selection-based explanations can be excluded. Continued investigation into the process by which multifamily groups form, as well as the study of the social behaviors within such groups, will provide a deeper understanding of social evolution in this system.

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