

Kin discrimination in the amblypygid, *Damon diadema*

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Abstract. Sociality occurs in less than one percent of all arachnids. Prolonged subsocial behavior with amicable mother-offspring-sibling associations that extend for a year has recently been reported in captive amblypygids, *Damon diadema* Simon 1876 (whip spiders; order Amblypygi, family Phrynichidae; Rayor & Taylor 2006). Many social animals have the ability to discriminate kin from other conspecifics so that benefits of group-living are preferentially directed toward kin, although kin discrimination is rare in social spiders. To aid in quantifying rates of behavior, we developed a behavioral ethogram of social and agonistic interactions in immature *D. diadema*. We conducted two experiments that demonstrate the ability of immature *D. diadema* to recognize and behave differentially toward kin. In a series of cross-introduction experiments, immature kin or non-kin were introduced into social groups to determine whether aggression and non-aggressive interaction rates varied based on their relationship to the resident animals. To test the hypothesis that differences in behavior were due to familiarity with the habitat, rather than with kinship with resident animals, individuals were cross-introduced into unfamiliar habitats with kin or non-kin. In these introduction experiments, kinship determined the level of aggression among individuals while habitat familiarity did not have an effect. Using olfactory cues alone in Y-maze choice experiments, 9-month old amblypygids discriminated their mother from an unrelated adult female and spent significantly more time near their mother. We discuss our results in relation to other examples of kin discrimination in insects and arachnids, and potential benefits to amblypygids at different ages.

Keywords: Whip spider, social behavior, kin discrimination, olfaction, ethogram

Although nestmate discrimination is well known among the social insects (e.g., Hölldobler & Wilson 1990; Vander Meer et al. 1998), it appears to be rare or absent in most of the subsocial and social arachnids (Lubin & Bilde 2007). Most social spiders readily accept unrelated individuals into colonies with no behavioral conflicts between kin and non-kin members of the colonies (D’Andrea 1987; Pasquet et al. 1997; Lubin & Bilde 2007). In most cooperative social spider species, immigration into the colonies is so rare and the costs of accepting non-kin into the group so minor, it has been hypothesized that the spiders have not evolved mechanisms for kin discrimination (Avilés 1997; Evans 1999). However, recent studies indicate that, although kin discrimination behavior is not expressed under normal circumstances in most social arachnids, at least some arachnids can differentiate between kin and non-kin. The social Australian huntsman spider, *Delena cancerides* Walckenaer 1837 (Sparassidae) that live in retreats under tree bark that are limited in size and availability selectively attack unfamiliar, non-kin that enter their colonies (Rowell & Avilés 1996; Yip & Rayor pers. obs.). In the social crab spider, *Diaea ergandros* Evans 1995 (Thomisidae), juveniles preferentially cannibalize non-kin in times of food scarcity, while subadult females cannibalize unrelated females and sibling males before immigrant males (a pattern that maximizes outbreeding opportunities) (Evans 1999). Similar preferential cannibalism of non-kin occurs in starved subsocial *Stegodyphus lineatus* Pocock 1898 (Eresidae) and social *Delena cancerides* (Sparassidae) spiders (Bilde & Lubin 2001; Beavis et al. 2007). Among the solitary spiders, there is some evidence that prior to dispersal in the third instar, individuals discriminate among siblings or familiar

individuals to reduce cannibalism within the brood (Anthony 2003). Among the non-spider arachnids, kin recognition has only been seen in the highly social pseudoscorpion, *Paratemnoides nidificator* Balzan 1888 (E. Tizo-Pedroso pers. comm.).

Given the emerging evidence for some level of kin discrimination abilities in spiders, we chose to investigate the issue of kin discrimination in the prolonged subsocial amblypygid, *Damon diadema* (Simon 1876) (Order Amblypygi). Amblypygids have generally been characterized as solitary and intolerant of conspecifics (Weygoldt 2000). However, recent work on captive *D. diadema* suggests that this species may live in prolonged subsocial groups (Rayor & Taylor 2006). In captivity, immature *D. diadema* remain closely associated and highly interactive with their mother and siblings for approximately one year until becoming sexually dimorphic at 11–15 months of age (Rayor & Taylor 2006). Prolonged associations within the social groups include active aggregation, high levels of tolerance, and frequent amicable tactile interactions with their antenniform first legs (“whips”) to neighboring individuals. Prior to sexual maturity, agonistic behavior within sibling groups or with the mother is rare and there is a strong tendency for immature siblings of all ages to closely associate with one another. *Damon diadema* are often found in cave habitats, where there may be multiple overlapping groups within a single cave (Weygoldt 2000). In such a habitat, we predict that if there are advantages to maintaining long-term associations with kin it would be beneficial for individual *D. diadema* to be able to determine whether another amblypygid was kin or non-kin. As recent evidence suggests that diverse organisms such as social insects and ground-dwelling squirrels use cues of familiarity to direct behavior appropriately (Dahbi & Lenoir 1998; Mateo 2004), we did not distinguish between familiarity and kinship in this study. As preferences for aggregating with kin have been consistently demonstrated in other social species (Krause &

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Table 1.—A list of the ages of members of each clutch during each observation or experiment. *Birth indicates the day when the amblypygids descended from their mother's abdomen and were freely mobile. The mother of clutches 1 and 2 died of natural causes prior to all but the baseline observations of behavior. The mother of clutch 3 remained with her offspring for the duration of the study.

Clutch	Date of birth*	Number in clutch	Mother's status	Age during experiments		
				Baseline observations	Introduction experiments	Olfactory recognition experiments
1	15 Apr 2002	13	Died April 2003	10–11 mo	15–17 mo	–
2	25 Dec 2002	8	Died April 2003	–	7–9 mo	–
3	8 Jan 2003	20	Alive	–	7–9 mo	9 mo

Ruxton 2002), we predicted that *D. diadema* would be able to discriminate between kin and non-kin, displaying higher levels of aggression towards non-kin and interacting more with kin animals.

To investigate kin discrimination in immature amblypygids, we conducted a series of cross-introduction experiments. We introduced familiar kin or unfamiliar non-kin individuals into social groups to determine whether aggressive and non-aggressive interaction rates varied based on their relationship to the resident animals. To test the hypothesis that differences in behavior were due to familiarity with the habitat, rather than kinship with resident animals, we also cross-introduced individuals into unfamiliar habitats with kin or non-kin. We developed a behavioral ethogram to aid in quantifying the social and agonistic interactions observed in immature amblypygids. Our second experiment tested the hypothesis that a young amblypygid is capable of distinguishing its mother from an unrelated adult female using only olfactory cues.

METHODS

Subjects.—*Damon diadema* (Order Amblypygi, Family Phrynichidae) are native to Tanzania and Kenya where they are found in caves or on buttressed trees in coastal forests (Weygoldt 1999). Adult females used in this study were wild-caught in the Usumbara Mountains of Tanzania and obtained through several dealers over a two-year period. Females were bred in the laboratory with wild-caught or first generation captive males. Due to time differences when the adult females were obtained, their source, and relative size (= age), we have no reason to believe that any of the adult females were related and know that none of the clutches shared fathers. Three clutches were used in this study; all were born and raised in captivity. Clutch refers to a cohort of same age siblings. Members of Clutches 1 and 2 were half-siblings by the same mother that were kept together for the duration of this study. The age of members of each clutch, the status of their mother, along with their ages during each experiment are given in Table 1.

To permit identification of individuals, young were individually marked on their carapace with Testor's enamel paint. Voucher specimens were deposited in the Cornell University Insect Collection and at the Smithsonian Institution National Museum of Natural History. Extensive video documentation of social interactions and behavioral responses during introductions are available through the Cornell University Laboratory of Ornithology, Macaulay Library. Video vouchers are archived in the Macaulay Library and can be found online at: <http://animalbehaviorarchive.org>. These videos can

be located through an Advanced Search of the Notes for "Rayor Amblypygid Sociality" or by species name, Rayor, and behavior.

Housing and Diet.—Amblypygids from Clutches 1 and 2, and from Clutch 3 were housed in glass aquaria measuring 50 cm × 26 cm × 42 cm. To simulate natural habitat conditions and accommodate the amblypygids' thigmotactic preferences, cork bark lined the walls of the aquaria. A soil/vermiculite substrate on the floor helped maintain humidity. Water was supplied in a water dish and misted onto the bark. Animals were fed live domestic crickets (*Acheta domesticus*) approximately twice weekly. They were maintained at room temperature (~ 25° C) and largely kept in the dark. Behavioral observations were made in the dark under red light using a Sony digital camcorder (model DV-TRV30 NTSC), with "nightshot" infrared lighting.

Behavioral repertoire.—Although behaviors involved in courtship and fighting have been described for some species of amblypygids (Weygoldt & Hoffmann 1995; Weygoldt 2000; Fowler-Finn & Hebets 2006), no comprehensive behavioral repertoire or ethogram has been published that describes the characteristic behaviors involved in social interactions among immature amblypygids and their mothers. An ethogram of key behaviors pertinent to social interactions was developed for *D. diadema* to quantify rates of behavioral interactions (Table 2). Behaviors associated with adult conflict or courtship were omitted. Descriptions of *D. diadema* behaviors were based on four years of observations from test subjects in this study and the larger number of individuals of all ages reported in Rayor & Taylor (2006).

Introduction experiments.—To assess rates of aggressive and non-aggressive interactions in an undisturbed situation, baseline observations of 12 immature individuals from Clutch 1 were observed for a total of 12 h. To evaluate the differences in behavioral responses of young amblypygids to familiar kin (K) and unfamiliar non-kin (N), and to familiar (FH) and unfamiliar habitats (UH), we introduced single focal individuals into social groups in each possible combination in 27 one-hour "introduction experiments" (Table 3). All kin were siblings (or half-sibs) that were familiar to one another, while all non-kin were unfamiliar with each other; we did not separate the effects of familiarity from kin discrimination based on other cues. In each introduction trial, one animal was removed from its natal group, left in isolation for a minimum of one hour and reintroduced into either its natal group or to an unrelated group. To distinguish between differences in the introduced individual's response to unfamiliar conspecifics compared to responses due to an unfamiliar "habitat" (cage), we also varied the locations where introductions took place. In

Table 2.—Behavioral repertoire involved in social and foraging interactions of immature *D. diadema*. Behaviors are divided into two categories: (a) those involving the whip and (b) those involving other parts of the body.

(b) Body postures, movements, and behaviors		
Behavior Name	Category	Description
Slow scan	N/A	Specific details of movement may vary, but whip movements are slow and context indicates that the animal is relaxed and carrying out routine scans of the area nearby.
Inspect	Interaction	Angle subtended by whip motion is small; whip movement is focused in a specific area, but whip does not necessarily contact another object or animal.
Touch whip	Interaction	The focal individual makes contact with another amblypygid's whip.
Touch other amblypygid	Interaction	An individual touches its whip(s) to any part of another amblypygid's body, excluding the whips or palps.
Touch palps of other	Interaction	The focal individual touches its whip(s) to any part of the palps of another amblypygid or to the nearby region (chelicera).
Touch by other	Interaction	Focal individual is touched by the whip of another amblypygid.
Touch non-amblypygid	Interaction	An individual makes whip contact with a non-amblypygid object (e.g., a prey item).
Flinch whip	Interaction, Aggression	The whip is briefly and quickly jerked, usually backward. Most commonly seen after animal contacts another amblypygid or prey item.
(b) Body postures, movements, and behaviors		
Behavior Name	Category	Description
Relaxed closed	N/A	Amblypygid's body is flat against the substrate, and palps are closed. Typical resting posture.
Relaxed open	N/A	Amblypygid's body is flat against the substrate, but palps are open. Contrasts with palp opening during aggression in that palps loosely drop open, often with the femur at $\sim 45^\circ$ hanging downwards. Occurs in relaxed contexts, as a typical resting posture.
Agonism low	Aggression	Palps are opened such that the angle between the femur and tibia is less than 90° . Right and left palps are usually in symmetrical positions. Palps typically open rapidly and are briefly held open before returning to the "relaxed closed" posture.
Agonism high	Aggression	Similar to Agonism low, but femoral/tibial angle is greater than 90° and typically held open for a significantly longer period. Indicative of a higher level of aggression.
Bat/swat palps	Aggression	One or both palps are opened and swung through the area in front of an amblypygid. The speed of this movement is generally not as fast as that seen when an individual is making a grab at another amblypygid or prey item. Possible context: locating another animal prior to making a grab at it.
Grab	Aggression	Both palps are moved simultaneously towards another animal. This movement is quick and clearly aggressive (possibly an attempt to remove the other animal's whip or leg). Movements are essentially the same as during prey capture, but context is interaction with another amblypygid.
Body elevated off substrate	N/A	Legs are straightened more than during typical resting posture, such that the amblypygid's body is no longer in contact with the substrate. May indicate heightened alertness; often seen in contexts of aggression or hunting.
Body pump	Aggression	Body is moved up and down as amblypygid bends and straightens its legs. Contexts are aggression and hunting.
Walk	N/A	Amblypygid walks across substrate, usually conducting whip scans as it moves.
Retreat	Aggression	Focal individual moves backwards, usually away from another amblypygid or prey item.
Erratic Run	Aggression	The animal moves across the bark at a speed faster than normal walking, periodically stopping and starting. Usually accompanied by quick movements of the whips in broad arcs. Animal often appears to be agitated, making efforts to quickly investigate its new surroundings.
Fast run	N/A	Usually seen in response to touching the base of an amblypygid's legs. The animal moves across or to the other side of the bark extremely quickly, apparently without stopping during the run to make whip inspections of its surroundings.
Orient to prey/prepare to attack prey	N/A	Palps are drawn back to open position—femoral/tibial angle usually is not more than 90° , but angle between palp and body is small. Both whips are usually in the area near the prey item, repeatedly and briefly touching the prey. The amblypygid's body is oriented facing the prey. This behavior is usually followed by grabbing at the prey.
Grab at prey	N/A	Both palps move synchronously from open position towards prey.

Table 2.—Continued.

(b) Body postures, movements, and behaviors		
Behavior Name	Category	Description
Capture prey	N/A	Prey is impaled on the metatarsal spikes, at the end of the palpal hands. If the first capture attempt is unsuccessful, an individual may make several additional grabs at the prey item.
Drop prey	N/A	Prey is released from palpal hands. May occur while prey is still alive, or after it has been killed; may be accidental or intentional.
Eat prey	N/A	Amblypygid holds prey in palpal hands during consumption, may move hands, and adjust position of prey. Chelicerae move up and down, masticating prey.
Groom	N/A	Includes several behaviors such as back and forth movements of the palps, drawing of the whips through the chelicerae, or chewing on the tip of the whips or legs with the chelicerae.

K-FH trials, the test subject was returned to its natal cage with its siblings who had remained undisturbed. In K-UH trials, the test subject's entire sibling cohort was moved to a novel cage with unfamiliar bark, allowed to adjust to the cage overnight and then the test subject reintroduced. In N-FH trials, the test subject's kin were removed from their natal cage temporarily, the unrelated cohort was introduced into the test subject's natal cage, allowed to adjust overnight, and the unrelated test subject introduced. In N-UH trials, the test subject was introduced to a cage of unfamiliar individuals who had not been disturbed otherwise. In each introduction trial, the introduced individual was observed for one hour immediately following transfer.

Observations were videotaped under red light, behaviors were recorded on a handheld Psion Workabout (a PDA), and later analyzed using Noldus Observer 4.0 behavioral software. Introduced individuals were observed using focal sampling and all reported behavior reflects those of the introduced individual. Behaviors were attributed to initiated behaviors (I) or received behaviors (R), although the focal sampling method does not clearly reflect the extent that most behaviors involving whip contacts were often reciprocal. We assessed interaction rates, using the following non-aggressive interactive behaviors between individuals: whip-whip touches (I or R), touching another individual's body or legs (I), touching the palps of another individual (I), getting touched by another amblypygid (R), whip flinches (R) (Table 2). To assess aggressive events, we included all behaviors that were consistently observed in aggressive contexts: body pumping (I), agonistic palp opening (both low and high levels of agonism – I), grabbing or swatting with palps (I), retreating (R), and erratic running (R). The aggressive behaviors ranged

from retreating to clear threats (body pumping or agonistic palp opening, erratic running away), to active aggression (grabbing or swatting with palps). Swatting or grabbing with palps is as serious as aggressive behaviors get among immature *D. diadema*. We have no direct or indirect evidence of immature individuals engaging in serious conflicts or cannibalism in this study or previous studies (Rayor & Taylor 2006). During observations for baseline and introduction experiments, the adult females were largely uninvolved in behavioral interactions with their offspring, so that they were excluded from these analyses.

For each introduction experiment we calculated the number of total, initiated, and received non-aggressive interactions and aggressive behavior by the introduced focal animal. There were so few received aggressive behaviors that only total aggression was statistically analyzed. Rates were calculated as the number of aggressive interactions per hour. To examine the effect of different sets of predictors on the response variables of aggressive or non-aggressive interaction rate four sets of 2-way ANOVA's were carried out. The best models included both kinship with the resident social group and familiarity with the habitat as predictors. For total, initiated, and received aggression and interaction rates, we tested for effects of kinship (kin (K) vs. non-kin (N)), habitat (familiar (FH) vs. unfamiliar (UH)), as well as the interaction between kinship and habitat. All aggression data were log transformed to achieve normality, while interaction data were normally distributed without transformation. To avoid complications associated with pseudoreplication, we minimized the number of times each individual was used in each series of experiments. The kin introduction experiments were designed such that the potential for animals from different cages to become familiar with each other was limited. Although young were involved as residents in multiple kin introduction trials, only two individuals from Clutch 2 were used as the introduced animal in more than a single trial (but not in the same experimental context).

Olfactory Recognition Experiments.—To determine whether olfactory cues play a role in discriminating familiar from unfamiliar females, we evaluated the behavioral response of immature amblypygids to olfactory cues from their mother and an unrelated adult female in an “olfactometer.” The vertically oriented Y-shaped olfactometer was composed of three 7.5 cm diameter clear acrylic tube arms connected through a “choice chamber” or a black 3-way plumbers joint

Table 3.—Design of the Introduction experiments, number of focal individuals observed and the code for each experiment. Kin were all familiar siblings or half-sibs, while Non-kin were unfamiliar and unrelated individuals. Each trial lasted 1 h.

Habitat	Animals	
	Kin	Non-kin
Familiar	K-FH <i>n</i> = 7	N-FH <i>n</i> = 7
Unfamiliar	K-UH <i>n</i> = 7	N-UH <i>n</i> = 6

with a clear plexiglass window (Fig. 1). The arms included the introduction arm (61 cm long) through which the test subject was introduced, and two 48 cm long choice arms. The introduction arm had a removable window through which the test amblypygid was introduced. The plastic floor of the tubes was overlaid with mesh screening to provide a gripping surface for the amblypygid to walk on. At the end of each choice arm, the test amblypygid's mother and an unrelated, unfamiliar adult female were in 8 cm long screened containers. A double layer of wire mesh screen separated the choice arm from the females' containers, allowing airflow over the females but preventing any physical contact (including whip contact) between the test subject and the females. Air was pulled through the olfactometer by a slow fan affixed to the end of the introduction tube, resulting in air flowing from the females' containers upwards through the choice chamber, and past the point of introduction. Within the introduction tube and choice chamber, the test subject was potentially exposed to chemosensory cues from both adult females, but likely only exposed to cues from a single female within each choice arm. The position of the mother and unfamiliar female were randomized between trials. All trials were conducted in the dark under red light. Between trials, the apparatus was cleaned with ethanol.

The test subject was able to wander freely within the olfactometer. During each trial, the duration of time that the amblypygid spent in each region (0–6) within the olfactometer was recorded. Region 4 was always used to designate the side of the apparatus containing the mother, regardless of whether the mother was on the left or the right side of the apparatus. Additionally, the number of times that an individual passed between regions was recorded. Thus, the olfactometer evaluated whether immature amblypygids oriented toward and spent more time near their familiar mother based on olfactory cues alone. Trials were conducted for 45 min. The experimental subjects included each of the 15 members of Clutch 3 when they were 9 months old. Test subjects were only used once. The mother of Clutch 3 and the same unfamiliar adult female were used in all trials. Wilcoxon signed rank tests were used to compare the time test subjects spent near the mother and near the unrelated female. Mean time spent in each region of the testing apparatus was compared using a Kruskal-Wallis test. General activity levels of test animals were measured by determining the number of times a test subject moved between regions during the trial.

RESULTS

Behavioral Observations.—The sensory and social lives of amblypygids are centered on the thin antenniform first pair of legs (or “whips”), which are extensively used for odor discrimination (Hebets & Chapman 2000), spatial location (Hebets 2002), and tactile contact between individuals (Rayor & Taylor 2006; Rayor 2007). The whips are covered with sensitive chemosensory and mechanosensory setae (Foelix et al. 1975; Foelix & Hebets 2001; Spence & Hebets 2006), and are capable of delicate movements approximately 340° around the horizontal axis of their bodies, as well as vertical movement above the body (pers. obs.). Both amicable and agonistic social interactions were mediated primarily through whip movements or whip contact between individuals,

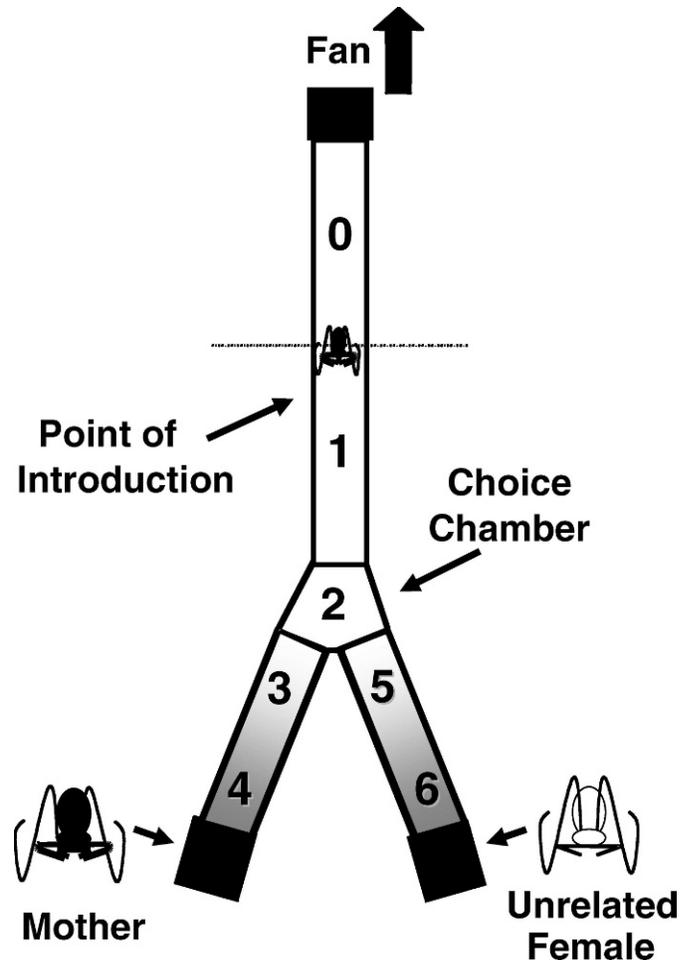


Figure 1.—Diagram of olfactometer with each of the numbered regions where the test amblypygid could move within the olfactometer. The fan pulled air from the adult females through the olfactometer. The choice chamber (Region 2) joined the three arms of the olfactometer and received odors from both females. The mother and unrelated female were separated from the main body of the olfactometer by a double layer of mesh screen.

although agonistic palp movements also played a role (Table 1). Particularly in social or novel situations, amblypygids' whips are in continuous motion exploring the environment and their neighbors. Animals frequently touch each other with their whips, speeding up whip motions when highly excited or agitated. Whips are moved in broad sweeps or in short localized taps. Palps are opened in the context of prey capture and aggression, and occasionally when an individual is relaxed and at rest. Specific positions of the palps and contextual information are almost always sufficient to determine whether an amblypygid is opening its palps in the context of aggression, predation, or relaxation (Table 2). Compared to overt whip and palp movements, changes in body posture were less apparent with the exception of dorsoventral pumping of the body and/or stiling of the legs, which indicated heightened aggression.

Introduction Experiments.—Baseline observations of undisturbed 11–12 month old siblings from Clutch 1 show a relatively low rate of aggressive and non-aggressive interactions (Fig. 2, 3). As all introduction experiments involved

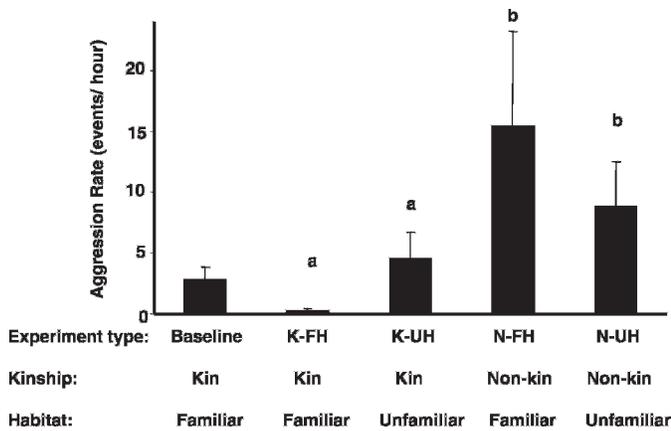


Figure 2.—Rates of aggression in each of the five treatments. Standard Error bars for total aggression are shown.

members of Clutches 2 and 3 when they were younger (7–9 months old), we only compared baseline observations with those of K-FH, which differed in the level of disturbance associated with removing and reintroducing a member of the group but not in kinship or familiarity with the habitat. There were no significant differences in interaction rates between these two groups. Aggressive behaviors were significantly more common during baseline observations ($F_{1,18} = 8.98$, $P = 0.0081$, Adjusted $R_2 = 22.0$) than K-FH. Because aggression rates increase as *D. diadema* become sexually dimorphic at 11–14 months (Rayor & Taylor 2006), we anticipated more aggression in the baseline observations than were observed.

In introduction experiments, resident animals rapidly interacted with the introduced individual. Compared to the behavior of residents, the behavior of the focal introduced animal was often hesitant; its movements jerkier and more agitated. Although interaction rates of the introduced subjects were relatively high in all experiments (events per hour: median = 144, range = 7–324), rates of aggressive interactions were consistently low among K-FH (events per hour: median = 0, range = 0–1) compared to N-FH (events per hour: median = 4, range = 0–51). Even among the focal individuals in N-UH, which experienced the most social disruption, aggression was primarily expressed through low level behaviors (body pumping and low agonistic palp opening). The low level aggression of immature *D. diadema* is in sharp contrast to the significantly more aggressive behaviors characteristically observed when unfamiliar adults are introduced to one another (repeated swipe palp, grappling, fencing position; Weygoldt 2000; pers. obs.) that may cause serious injuries or result in cannibalism.

Young amblypygids recognize non-kin and behave differentially toward them based on introduction experiments (Fig 2). The best aggression rate model included animals and habitat as predictors (2-Way ANOVA: Aggression: $F_{2,26} = 4.045$, $P = 0.0306$, adjusted $R_2 = 19.0\%$). Kinship determined the rate of aggression involving the introduced individual (Kinship: $F_{1,26} = 6.96$, $P = 0.0144$), but familiarity with the habitat had no impact ($F_{1,26} = 1.3$, $P = 0.25$). Aggression rates were significantly higher for non-kin than kin (Least Square Means \pm SE: Kin = 0.702 ± 0.307 ; Non-kin = 1.85 ± 0.32).

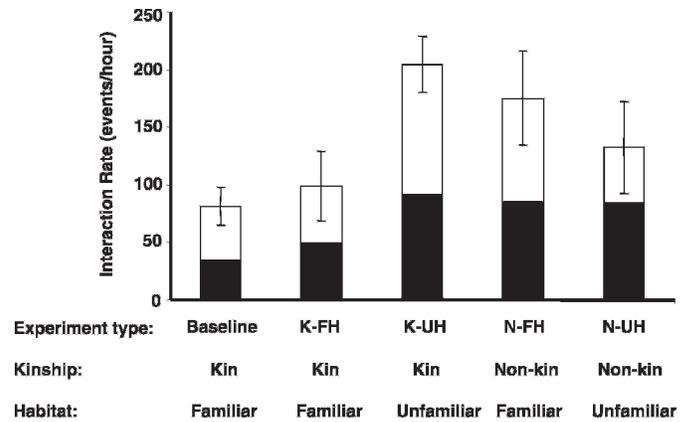


Figure 3.—Rates of non-aggressive interactions in each of the five treatments divided into initiated behaviors (black) and received behaviors (white). Standard Error bars for total interactions are shown.

No aspect of non-aggressive interactions (total, initiated, or received) differed significantly among introduction experiment treatment groups in 2-Way ANOVA comparisons (Fig 3). Interaction rates were relatively high with 1.65 to 3.4 events per minute, primarily due to whip-whip touches, but did not significantly vary between treatment groups.

Olfactory Recognition Experiments.—Immature amblypygids were able to differentiate between their mother and the unrelated female using only olfactory cues. Most of the experimental individuals were active, extensively exploring the olfactometer. Twelve of the fifteen immature amblypygids left the introduction tube and choice chamber to explore one or more of the choice arms that had their mother or an unrelated female at either end. Over the entire 45-minute trial young from Clutch 3 spent significantly more time in the distal segment of the choice arm (Region 4) closest to their mother than in the area (Region 6) closest to the unrelated female (Wilcoxon signed rank test: $T_+ = 62$, $n = 12$, $P < 0.0386$). However, there was no difference between the total time spent in the mother's entire arm (Regions 3 and 4) compared to the unrelated female's arm (Regions 5 and 6) (Wilcoxon signed rank test: $T_+ = 49$, $n = 12$, $P = 0.2349$). In the initial movement into the choice arms equal numbers moved toward the mother and the unrelated female ($n = 6$ each). Three young explored only the mother's side of the olfactometer; nine explored both the mother's and unrelated female's sides. None of the experimental subjects explored only the unrelated female's side of the olfactometer. The majority of the focal individuals actively explored the olfactometer; the mean number of region changes over the duration of the trial was 14.93 ± 7.69 . There were no differences in the mean number of region changes based on whether an animal moved to the mother or unrelated female's region first (Mann-Whitney U-test: $U = 18.5$, $n_{\text{mom}} = 6$, $n_{\text{unrelated female}} = 6$, $P = 0.937$) or spent more total time near the mother (Region 4) or the unrelated female (Region 6) (Mann-Whitney U-test: $U = 20.5$, $n_{\text{mom}} = 8$, $n_{\text{unrelated female}} = 4$, $P = 0.461$). Together these results suggest that olfactory cues are sufficient for kin discrimination and that once the young had located their mother the familiar stimulus was sufficient to arrest their movements.

DISCUSSION

Our results suggest that immature *Damon diadema* respond differentially to kin and non-kin in the contexts of encountering unfamiliar individuals and in being more attracted to the olfactory cues from their mother. In all introduction experiments, the introduced nonkin individuals were significantly more aggressive than introduced kin. Although all individuals in a group were disrupted when moved to an unfamiliar habitat, familiarity with the habitat did not consistently affect the introduced individual's level of aggression compared to kinship. Our low sample sizes in this experiment could have resulted in an underestimate of the effects on behavior of manipulating the habitat. However, in the field, immature amblypygids almost certainly encounter unfamiliar individuals of all ages and potential threat, while most clutches are unlikely to be moved communally into unfamiliar habitats.

In olfactory recognition experiments, immature *D. diadema* were capable of discriminating their mother from unfamiliar adult females using only olfactory cues. As these results are based on the behavior of only a single clutch, further research will be required to document the full role of olfaction in kin discrimination in amblypygids. Hebets & Chapman (2000) have demonstrated that amblypygids are much more attuned to chemosensory cues than the other arachnids. In the field, adult male amblypygids (*Phrynus parvulus* Pocock 1902) use olfactory cues to locate reproductively mature adult females Hebets (2002). So it is not unexpected that these cues may be used in a social context in amblypygids. Use of olfactory cues for kin recognition is widespread in eusocial insects (Vander Meer et al. 1998), but has not been demonstrated in social spiders probably due to minimal costs associated with a lack of kin recognition in most of these social groups (Lubin & Bilde 2007). Amblypygids actively moving around while foraging at night are more likely to come in contact with unrelated animals than web-based social spiders foraging within the confines of their web. Among the spiders, olfaction has been demonstrated primarily in the sexual attraction of male to female spiders using short-range volatile cues on silk (Schulz 2004; Gaskett 2007).

Kin recognition abilities have been proposed to be advantageous in permitting nepotism among group members and for inbreeding avoidance (Sherman et al. 1997). Spatial overlap among individuals from several *D. diadema* clutches may occur within certain habitats, such as caves. The conundrum is to determine the context in which kin recognition is valuable to young amblypygids living in prolonged subsocial groups. Unlike the obvious advantages of group-living in the other arachnids (e.g., cooperative capture and sharing of prey, communal construction of retreat or web), Rayor & Taylor (2006) found few benefits of sociality in *D. diadema* beyond maternal defense of immature offspring. In the laboratory, sibling competition over prey is minimal (Rayor & Taylor 2006), although competition may be more intense in the wild. While there may be microclimate or warning advantages when young siblings pack together into small crevices during the day as they do in the laboratory, it is improbable that suitable crevices are so limited that there is the need to exclude non-kin from these sites in the wild. Nor does the need for kin recognition appear to be generated by

aggressive behavior of the adult females: In the laboratory, two mothers with clutches readily accepted non-kin young (close in age to their own offspring) who were introduced into their cages, and the mothers were amicable to their own subadult offspring after a separation of two months (unpublished data).

The most parsimonious explanation for kin recognition in *D. diadema* is that while immature animals are capable of kin recognition, the ability to recognize kin pays dividends once they disperse from the natal social group and encounter, or become, aggressive adults themselves. The only amblypygid which has been observed extensively in the field is *Phrynus parvulus* whose adults can be both territorial or wander widely within the habitat (Hebets 2002). For adult *D. diadema*, similar movements leading to encounters with other amblypygids are probable. The low level aggression of immature *D. diadema* is in sharp contrast to the aggressive fights observed when unfamiliar same-sexed adults encounter one another (Weygoldt 2000; Fowler-Finn & Hebets 2006). We hypothesize that the ability to recognize kin, using a variety of cues, may help reduce serious conflicts among familiar amblypygids later in life. We speculate that if adult amblypygids recognize their siblings as adults, this will result in fewer injuries within kin groups and aid in inbreeding avoidance. Further research on the role of kin discrimination and social behavior in amblypygids will broaden our understanding of a taxon better known for its solitary and aggressive behaviors.

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