

Aliens among us: nestmate recognition in the social huntsman spider, *Delena cancerides*

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Abstract Unlike all other social spiders, the social huntsman spider, *Delena cancerides*, has been reported to rapidly respond to non-nestmates with lethal aggression, similar to the behavior of some eusocial insects. We tested for the presence of nestmate recognition in *D. cancerides* under laboratory conditions by introducing 105 unrelated alien conspecifics into foreign colonies and comparing their behavior to 60 control spiders removed and returned to their natal colony. Spiders demonstrated nestmate recognition by investigating alien spiders far more than nestmates and by resting closer to nestmates than to aliens. Serious attacks or deaths occurred in 23% of all trials; however, aggression was not directed significantly more toward aliens than to nestmates. Most notably, aggression was largely mediated by the adult females (resident or alien), who were most likely to attack or kill other subadult or mature individuals. Young individuals (resident or alien) were largely immune from serious aggression. Spiders recently collected from the field tended to be more aggressive than spiders born and raised in the laboratory, possibly due to blurring of recognition cues related to laboratory husbandry. Our findings support the prediction that nestmate recognition should evolve when there is a benefit to discriminating against non-kin, as in this social

spider system where foraging individuals may enter a foreign colony and the colony retreat is a limited resource.

Keywords Social spiders · Nestmate recognition · Aggression

Introduction

The ability to discriminate kin from other conspecifics is a common trait among social animals, as it allows altruistic behaviors to be preferentially directed toward kin (Hamilton, 1964; Hölldobler and Wilson, 1990; Holmes, 2004). Most eusocial insects recognize their nestmates, with the resolution of kin discrimination at the level of the colony (Breed et al., 1994; Clément and Bagnères, 1998; Strassmann et al., 2000; Tarpay et al., 2004). While nestmate recognition is common among the eusocial insects (Wilson, 1971; Singer and Espelie, 1992; Clément and Bagnères, 1998; Vander Meer and Morel, 1998) nestmate recognition is rare or absent in the subsocial and social arachnids (Lubin and Bilde, 2007).

There is a continuum of social behavior in the spiders from small subsocial mother–offspring–sibling groups to complex, cooperative societies of thousands of individuals (Buskirk, 1981; Avilés, 1997; Lubin and Bilde, 2007). Most social spiders, despite multiple evolutionary origins, share a suite of traits that includes the acceptance of alien spiders (unrelated and unfamiliar conspecifics) into the group without overt aggression (Lubin and Bilde, 2007). These social spider species do not appear to differentiate between conspecific aliens and members of their own colony (Pasquet et al., 1997), silk from kin or non-kin (Bilde et al., 2002; Buser, 2002), or even heterospecific from conspecific spiders in the same genus (Seibt and Wickler, 1988a).

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Most social spiders may have never evolved nestmate recognition because the costs of sharing resources are relatively small, and non-relatives are encountered only rarely. While there is undoubtedly competition for resources within social spider colonies (Ward, 1986; Seibt and Wickler, 1988b; Avilés and Tufiño, 1998; Bilde et al., 2007), the benefits of group-living may mitigate the costs of sharing resources (Rypstra, 1989; Avilés, 1997; Uetz and Hieber, 1997; Avilés and Tufiño, 1998; Jones and Parker, 2002; Whitehouse and Lubin, 2005). Migration among colonies is rare in most cooperative spiders, and in a number of species, extreme inbreeding is the norm, suggesting that these spiders encounter aliens rarely (Avilés, 1997; Lubin and Bilde, 2007).

Of the social spiders, only the Australian huntsman spider, *Delena cancerides* Walckenaer (Sparassidae), has been reported to rapidly attack alien conspecifics (Rowell and Avilés, 1995; Beavis et al., 2007). When aliens were introduced into laboratory colonies, colony members killed and partially ate aliens, typically within 24 h (Rowell and Avilés, 1995). In addition to aggression among adult females, both adult males and juveniles (all juveniles were unsexed) were killed, and both adults and juveniles killed aliens. However, preliminary experiments by L. Rayor indicated that adult males and small juveniles were usually accepted into the colony, while most aggression was directed toward reproductive or subadult females. A recent study on kin-recognition in *D. cancerides* supports these preliminary results, showing that adult females usually accept non-kin juveniles if they are small enough but kill older juveniles (Beavis et al., 2007). While the extent of the aggression toward non-kin seen in *D. cancerides* appears to be unique among the social spiders, preferential cannibalism of non-kin has been demonstrated in a few other spider species. Spiders preferentially cannibalize non-kin after several days or weeks of starvation in two subsocial species (Evans, 1999; Bilde and Lubin, 2001) or shortly after mother and offspring disassociate in two species of solitary wolf spiders (Anthony, 2003; Roberts et al., 2003).

The ecology of *D. cancerides* differs dramatically from other social spiders and is predicted to favor the evolution of nestmate recognition. *Delena* is one of only two social spider genera with spiders that do not live in connected or communal webs (Evans, 1995; Rowell and Avilés, 1995; Avilés, 1997). Spiders of the other non-web building social spider genus, *Diaea*, construct expandable retreats of leaves and silk (Evans, 1995). In contrast, *D. cancerides* spiders live exclusively under tree bark. Whereas the acceptance of immigrants into a web-based spider colony may increase the colony's total web area and prey capture capacity, the relatively small *Delena* retreats cannot be created or expanded by the spiders (Rayor et al., in prep.). Benefits associated with living under the retreat (protection

from abiotic elements, defense from predators, prey sharing by younger animals, etc.) are unlikely to increase with the addition of immigrants. Moreover, as only a single adult female typically reproduces per colony, older immigrant females are potential reproductive competitors of the breeding female or her daughters. In suitable habitats, *D. cancerides* colonies may be as close as 1 m apart, and some single trees house multiple distinct colonies (Rayor et al., in prep.). Unlike all other web-based social spiders, these spiders are central place foragers, leaving the retreat at night and returning at dawn. Spiders move as far as 10 m on foraging bouts (E. C. Yip, unpubl. data). It is therefore highly probable that conspecific aliens are encountered in the field. If accepting immigrants into the colony imposes a potential cost to the residents—or some portion of them—we predict that individual *D. cancerides* spiders will discriminate between nestmates and aliens.

To test the prediction that members of *D. cancerides* colonies discriminate nestmates from alien conspecifics, we introduced individuals of different ages and sexes into foreign colonies in the laboratory. The explicit behaviors indicative of nestmate recognition are difficult to characterize. The reactions of eusocial insects to alien intruders range from accepting the alien while partially withholding food resources, to frequent investigative touches, to outright attack (Wilson, 1971; Hölldobler and Wilson, 1990; Pearce et al., 1990). Other eusocial insects apparently lack colony level recognition (Clément and Bagnères, 1998; van Wilgenburg et al., 2007). In light of these highly variable responses, we evaluated both overt aggression and more subtle behaviors that may differ between nestmates and aliens. We compared interactions with aliens to those with nestmates, taking any differences in behavior to be indicative of nestmate recognition. Finally, we examined how the age and sex of the alien and characteristics of the host colony correlate with the probability of aggression.

Materials and methods

Study organism and care

Delena cancerides is endemic to southern Australia and Tasmania (Main, 1962). Spiders form colonies of up to 300 individuals under the bark of *Acacia*, *Eucalyptus*, *Callitris* and *Casuarina* trees (Rowell and Avilés, 1995; Rayor et al., in prep.). Most colonies consist of a single adult female with multiple cohorts of immature offspring living together although colonies with 2–3 adult females are occasionally found in the field (Rayor et al., in prep.). Only a single female successfully produces egg sacs at a time within a colony (unpubl. data). Spiders live for ~2.5 years and typically reach sexual maturity in 10 or 11 instars. We

considered the large-bodied seventh through ninth instars whose sexes can be readily distinguished to be subadults. Colonies were collected from 10 sites in southern Australia (2 in the Australian Capital Territory, 6 in New South Wales, 1 in South Australia, and 1 in Victoria). While chromosomal arrangements may differ considerably among *D. cancerides* populations from different areas in Australia, all interbreeding, morphological, and molecular evidence indicates that these spiders remain a single species (Sharp and Rowell, 2007).

Colonies used in this study consisted of either third or fourth generation descendents of spiders collected from the field in January–March 2002 (here termed ‘laboratory’ colonies), or spiders recently collected, either in January–March 2002 for trials conducted in 2002 or February–April 2006 for trials conducted in 2006 (here termed ‘wild’ colonies). Spiders were housed in glass terraria with total surface areas of 2,743 or 4,888 cm². To replicate their retreats under tree bark, clear 3 mm thick Plexiglas sheets were attached 1–2 cm away from the long sides of the terraria with layered squares of Velcro, creating thigmotactically appealing retreats of 504 or 888 cm² that are consistent with retreat sizes found in the field (Rayor et al., in prep.). Colonies with more and/or larger individuals were housed in larger terraria. Substrate at the bottom of the terraria was a mixture of soil and vermiculite. Each colony had a shallow water dish. Colonies were maintained at room temperature (22–26°C) and approximately 12-h light and dark cycles prior to introduction trials; during the first day of trials, colonies were exposed to constant light so that experimental spiders could be easily identified on film. *D. cancerides* is nocturnal in the wild, yet spiders in the laboratory exhibit the same behavioral repertoire in the light as they do in the dark (e.g. feeding, mating, laying eggs, and all behaviors quantified in this study, unpubl. data). Colonies were fed 1 cricket (*Acheta domesticus*) or housefly (*Musca domestica*) per spider 1–2 times per week prior to introductions and again immediately preceding an introduction trial to standardize hunger levels.

Introduction experiments

We define ‘aliens’ as conspecific spiders introduced to the terraria of unfamiliar, unrelated colonies. ‘Controls’ refer to colony nestmates or kin, of the same age (within one instar) and sex as the alien, which were removed and returned to their natal colony during introduction experiments. The effects of familiarity and kinship are confounded in this study; however, the distinction between the two is mechanistic, with kinship being a form of ‘allele recognition’ or ‘phenotype matching’ and familiarity being recognition by ‘prior association’ (Holmes, 2004; Mateo,

2004). In this study, we were not concerned with the mechanisms maintaining nestmate recognition, but rather the characterization of behaviors that might indicate the presence of nestmate recognition. We report 15 experimental trials in 2002 and 90 experimental trials in 2005/2006.

In 11 of the 15 2002 trials, only alien spiders, third instar to subadult females, were introduced into foreign colonies composed of an adult female and one or two cohorts of young. In the remaining four trials, an adult female, a subadult female, an adult male and a subadult male were paired with a nestmate of the same age and sex that had been removed from its natal colony 1–2 months prior. Each pair of spiders was introduced into a colony that had a resident adult female. Spiders were placed in vials that were then opened inside terraria, and the spiders moved out of the vials of their own volition. Survivorship was tracked for 1 week, but all deaths occurred within 24 h. Survivorship data for the 2002 trials were qualitatively similar to those for the 2005/2006 trials, in terms of the frequency of attack and the age of spiders attacked. Therefore, these data were added to our analyses; however, because paired nestmates were separated from their natal colony for long periods of time, these data are not included in our comparisons between aliens and controls.

We conducted 90 experimental introductions from September 2005 to July 2006. Colonies were classified as either wild or laboratory colonies, and as having an adult female (‘AF colonies’) or lacking an adult female (‘no-AF colonies’). The origin of the colony (laboratory or wild) determined the classification of the trial. Aliens were classified as adult females, adult males, or immature spiders (unsexed juveniles third to sixth instar or sexed subadults seventh to ninth instar), and assigned an instar (age) based on body size (see Table 1). Aliens were paired with controls in 60 trials; no controls were used in 30 trials because colonies in these trials did not contain a nestmate of similar age and the same sex as the alien.

Experimental trials in 2005/2006 followed the following protocol: we lightly sedated alien and control spiders with CO₂ and weighed them on a Mettler Toledo scale (AG285). For each spider, we used Spi 2000 calipers to measure the maximal width of the cephalothorax and length of the second leg (the longest leg), from the tip of the tarsus to the coxa. Using a toothpick, Testors enamel paint was applied to the ventral and dorsal surfaces of the cephalothorax, taking care to leave the eyes, mouthparts, and book lungs unobstructed. Once the experimental spiders were fully recovered from the effects of the CO₂, they were simultaneously and gently coaxed onto the Plexiglas retreats. In all, the measuring and marking process took 15 min or less. An effort was made to minimize disturbance to the colony

Table 1 Sample size for introductions with different ages and sexes of spiders

Alien introduced	Presence of adult female	Year					
		2002		2005/2006			
		Wild		Laboratory		Wild	
		Alien only	Alien + kin	Alien only	Alien + control	Alien only	Alien + control
Adult female	AF	0	1	0	10	0	4
Adult female	No-AF	0	0	3 ^a	3	5 ^a	2 ^a
Immature	AF	11	2	9	9	0	7
Immature	No-AF	0	0	5	13	0	5
Adult male	AF	0	1	5	5	3	2
Total		11	4	22	40	8	20

AF indicates the presence of a resident adult female in the colony; No-AF indicates the absence of a resident adult female

^a One trial in each of these three categories involved the disappearance of spiders from the colony, and no aggressor could be identified. These trials are not included in aggression comparisons but are still included in all other measures

as experimental animals were introduced. We directly observed and recorded behavior for the first hour. Thereafter, terraria were video taped for 15 h on a 30 s interval, using one of two types of Sony digital video cameras (DCR-TRV900, DCR TRV30), to track behavior and survival of the experimental spiders. While the 30 s interval recording undoubtedly missed some short interactions, more intense confrontations, including killing and/or cannibalism take longer than 30 s, ensuring that these major events were recorded. For 3 days following the initial introduction, we measured nearest neighbor distance and survival for the control and alien once a day at approximately 24-h intervals.

We recorded behavior defined by the *D. cancerides* ethogram previously developed by L. Rayor and R. Walsh (unpubl. data). We recorded both of the experimental spiders' initial reactions in the first 5–10 s of the trial as calm (no movement or slow walking) or as frantic (erratic running). Throughout the study we recorded three primary types of contact: 'face-offs' where spiders face each other with first and second legs touching the other and often circle around one another with legs entwined and bodies held at a distance (note: face-offs occasionally preceded an attack by one participant on the other), 'touches' where spiders rapidly touch or tap any part of another spider's body, and 'aggregation' where spiders sit in contact with others for >1 min.

Colony demographics varied naturally, but to minimize colony disruption, we made no attempt to standardize spider density. Because alien and control spiders were introduced into the same colony simultaneously, spider density cannot account for differences in responses between aliens and controls. However, spider density was considered as a factor in explaining overall patterns of aggression.

Analyses

Because the number of colonies with suitable age-groups was limited, 26 of a total of 59 colonies were used more than once, depending on the age range of the spiders. No colony was used more than 5 times. However, to make trials as independent as possible, no spider was ever used twice as an alien. We further use colony as a random effect where possible.

We compared continuous responses (contacts and nearest neighbor distances) between aliens and controls using Wilcoxon signed rank tests because our data were not normally distributed. Binomial responses (aggression, initial reactions, whether spiders rested in contact) by aliens and controls were compared using a generalized linear model (GLM) with a binomial distribution. To account for the pairing of alien and control spiders within trials and subsequent correlation among data, the standard errors were corrected by a generalized estimator equation (GEE) with the trial as the repeated subject. Nestmate status (alien or control) was the explanatory variable. Sex, age, and their interactions with nestmate status, in addition to collection region, spider density, the status of the colony as wild or laboratory, and whether the alien was from the same collection region or a different collection region as the residents were also included in the models as possible variables of interest. These parameters were removed from the model if they failed to explain a significant portion of the variance.

We used mixed models, with colony as a random effect, to examine the relationships between nearest neighbor distance and sex and age. We used contingency tables and likelihood ratio tests to examine the correlations between the colony characteristics (laboratory or wild; with or without an adult female; type of alien) and aggression. Not

all trials yielded results for all measures, so separate sample size is reported for all analyses. SAS was used for GLM and GEE analyses. All others were conducted with JMP.

Results

Aggression

Out of all 105 trials from all years, 24 (23%) resulted in either the alien or control experiencing aggression. Most aggression was mediated by adult females. Of 24 trials with aggression, 18 (75%) involved adult females as aggressors, and an additional two involved subadult (eighth instar) females.

Of the 11 alien-only introductions in 2002, three spiders (27.3%; a sixth instar and two subadult females) were killed within 24 h. All aliens fifth instar and younger survived introductions, as well as two seventh instar females. All but one alien that survived rested with colony members after one day; the other alien rested with the group after 2 days. In the four paired kin and non-kin trials of 2002, all kin survived. However, the alien adult female was attacked and injured, while the subadult female, subadult male, and adult male survived without major incident.

Of all 90 trials (both paired with controls and unpaired) done in 2005 and 2006, three trials resulted in missing and presumed eaten nestmate spiders that could not be attributed to a specific aggressor (see Table 1). Of the remaining 87 trials, 19 (22%) resulted in either the alien being killed or attacked ($n = 11$ trials) or the alien killing or attacking a colony member ($n = 8$). Ten of 60 paired trials (17%) resulted in the control being attacked or attacking another individual. In six of these ten trials, aggression occurred between the two introduced animals, not another member of the colony. When aggression occurred between adult female aliens and their paired adult female control, it was difficult to distinguish whether the aggression indicated the exclusion of a non-nestmate or competition among adult females to secure the retreat as a breeding site, as there usually is only one adult female per colony. To be conservative, in these cases both the alien and control were designated as experiencing aggression. GLM analysis correcting for paired aliens and controls within trials by GEE, showed that aggression was not more directed toward aliens than controls ($n = 136$; alien/control: $z = 0.92$, $P = 0.36$) but that older spiders experienced increased aggression (age: $z = 2.45$, $P = 0.014$), as did spiders in wild colonies (laboratory/wild: $z = 3.09$, $P = 0.005$). Higher spider density increased the rates of aggression (spider density: $z = 2.78$, $P = 0.002$). Other parameters were not significantly related to the occurrence of

aggression, including introduced spiders' sex, collection region, or whether the alien was from the same collection region or a different collection region as the residents.

Initial reactions

Aliens tended to behave more frantically than controls in the initial 5–10 s of each introduction trial. Of 82 aliens introduced, 34 (41%) had an initial frantic reaction while only 14 of 55 controls (25%) did. Both age and nestmate status (alien or control) approached significance when considered together (GLM corrected by GEE: $n = 138$; alien/control: $z = 1.91$, $P = 0.057$; age: $z = 1.78$, $P = 0.075$). Nestmate status became significant if age was removed from the model (GLM corrected by GEE: $n = 138$; alien/control: $z = 1.99$, $P = 0.046$).

Contacts

Introduced spiders engaged in three major forms of contact (touch, face-off, and aggregation) with colony members. In 60 trials, aliens and controls differed depending on whether contact was received or initiated by the introduced spider (Fig. 1). When all three forms of contact were summed, aliens were subjected to significantly more contact than controls (Wilcoxon signed rank test: $T = -226.5$, $P = 0.018$), and most of this difference was from contact received as opposed to contact initiated (contact received: $T = -232$, $P = 0.007$; contact initiated: $T = -90$, $P = 0.36$). This effect was driven largely by a difference in touches received by aliens ($T = -195.5$, $P = 0.012$). Aggregation received and face-offs received were also greater for aliens than controls, but the differences were not significant (aggregation: $T = -17$, $P = 0.09$; face-off: $T = -1.5$, $P = 0.5$). No measure of initiated contact differed between aliens and controls (touch: $T = -90.5$, $P = 0.309$; aggregation: $T = -25$, $P = 0.27$; face-off: $T = -1.5$, $P = 1.0$). However, aliens tended to aggregate (spiders remained in relatively inactive contact for >1 min) more than controls during the first hour regardless of which spider initiated the contact ($T = -43$, $P = 0.020$).

Nearest neighbor distance

The effect of increased aggregation by aliens did not persist beyond the first hour. Over the 3 subsequent days (distance measured once per day), controls averaged only 6.1 cm away from their nearest neighbor, compared to 7.7 cm for aliens ($T = -121.5$, $n = 42$, $P = 0.028$). Average nearest neighbor distance, for both aliens and controls, significantly increased with age but showed no relationship with respect to sex. (Using a mixed model with age and sex as variables, colony as a random effect, and with colony

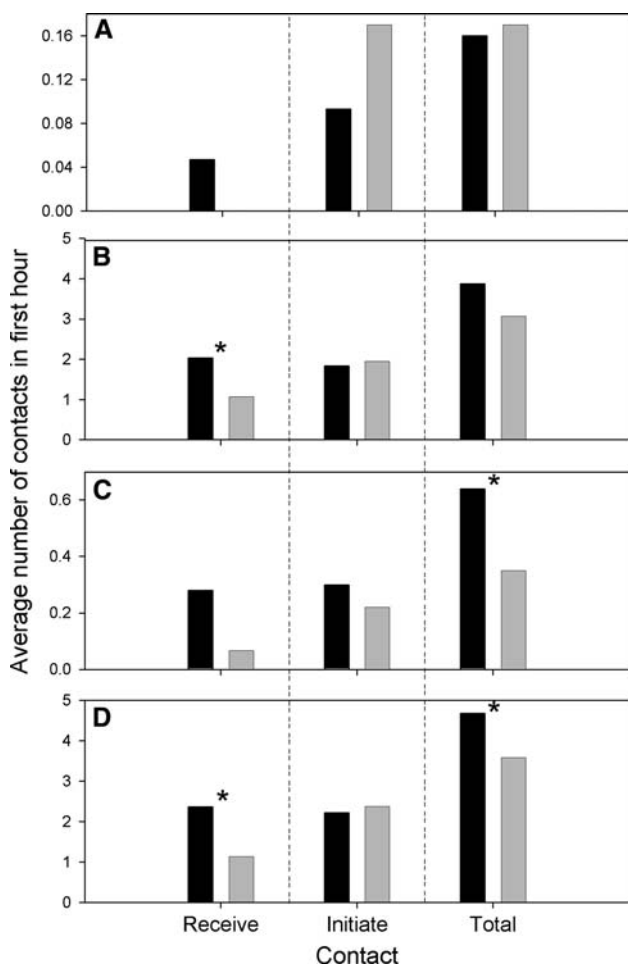


Fig. 1 The average number of contacts received, initiated, and in total for aliens in *black* and for control spiders in *gray*. Contact is subdivided into **a** face-offs, **b** touches, **c** aggregations, and **d** total summed contact. Asterisk denotes significance

origin (laboratory/wild) and nestmate status (alien/control) as covariates: adjusted $R^2 = 0.53$, $n = 80$; $\ln(\text{age})$; $F = 11.0$, $P = 0.0014$; no significant interactions: $\ln(\text{age}) \times \text{alien/control}$ $F = 2.89$, $P = 0.094$; $\ln(\text{age}) \times \text{laboratory/wild}$ $F = 0.60$, $P = 0.44$; sex $F = 0.78$, $P = 0.38$; no significant interactions: sex \times alien/control $F = 0.55$, $P = 0.46$; sex \times laboratory/wild $F = 0.50$, $P = 0.48$). Controls were more likely to rest in direct contact with other spiders than were aliens during the initial 3 days, as were younger spiders (GLM corrected by GEE: $n = 110$; alien/control: $z = 2.69$; $P = 0.007$; age: $z = 3.76$, $P = 0.0002$).

Wild versus laboratory reared spiders

As indicated by the aggression analysis, spiders from colonies that had recently been captured in the wild were significantly more aggressive than individuals born and raised in the laboratory (Table 2). Adult females introduced

into wild colonies were more likely to experience aggression than those introduced into laboratory colonies, either by fighting with the resident adult female or by attacking and sometimes consuming juveniles if a resident adult female was absent. Males tended to be attacked more in wild than laboratory colonies, but this was not significant with a Bonferroni correction. Immature spiders experienced similar aggression when introduced into both wild and laboratory colonies. Control spiders experienced similar aggression levels regardless of whether they were from wild or laboratory colonies. However, there was a non-significant trend for adult female controls to experience more aggression if they were from wild colonies (Table 2). This trend was due to the control adult female attacking the alien, rather than from conflict between the control and the rest of the colony.

Discussion

Do Delena cancerides spiders recognize nestmates?

Our results support the hypothesis of nestmate recognition in *D. cancerides*. Spiders were able to differentiate alien from control and showed increased investigative contact toward alien spiders. Increased contact toward unfamiliar animals has been found in a variety of taxa, including ground squirrels (Mateo, 2002), voles (Fadao et al., 2000), and ants (Dahbi and Lenoir, 1998). The reason for this pattern may be that unfamiliar recognition cues take longer to process and match to a ‘template’ (Mateo, 2004). Alien spiders also rested farther away from colony member than controls on average, reflecting an absence of integration into the colony.

We detected a marginally significant difference in initial reactions (frantic running or calm) between aliens and controls. Our behavioral assay is likely to be conservative in indicating stress caused by relocation into an unfamiliar colony. While frantic running clearly demonstrates heightened excitement, resting perfectly still may reduce the probability of attracting the attention of a hostile adult female. Thus, some outwardly calm spiders may have been stressed. Nevertheless, this result is consistent with our other results showing spiders can differentiate aliens from nestmates.

Aggression comparisons

In contrast, we found little evidence that aggression is primarily directed toward aliens. Aliens were not subjected to more aggression than controls, and an individual’s age strongly influenced the probability of aggression. Because aggression was most common among adults, particularly

Table 2 The percentage of trials involving aggression in laboratory ('lab') and wild spiders for each type of introduction

Introduced Spider	Spider/female presence	Lab or wild	Aggression (%)	χ^2 (df)	<i>n</i>	<i>P</i> value	
Spider/female presence designates the age and sex of spider introduced, adult female ('Female'), immature, or an adult male ('Male') and the presence ('AF') or absence ('No-AF') of a resident adult female in the colony	Aliens	Female/AF	Lab	30.00	8.51 (1)	10	0.0035*
			Wild	100.00		5	
		Female/No-AF	Lab	0.00	8.46 (1)	5	0.0036*
			Wild	80.00		5	
		Immature/AF	Lab	11.11	0.57 (1)	18	0.45
			Wild	19.05		20	
		Immature/No-AF	Lab	11.11	1.03 (1)	18	0.31
			Wild	0.00		5	
		Male/AF	Lab	0.00	7.013 (1)	10	0.0076
			Wild	50.00		6	
	Controls	Female/AF	Lab	30.00	3.51 (1)	10	0.061
				Wild		80.00	
		Female/No-AF ^a	Lab	0.00	0.00 (1)	3	–
			Wild	0.00		1	
		Immature/AF	Lab	0.00	0.00 (1)	9	–
			Wild	0.00		9	
		Immature/No-AF	Lab	7.69	0.50 (1)	13	0.48
			Wild	20.00		5	
		Male/AF	Lab	0.00	2.21 (1)	5	0.14
			Wild	33.33		3	

* Indicates significance, accounting a Bonferroni correction, yielding a maximum *P* value threshold of 0.00625 to achieve an α of 0.05 for the combined eight tests

^a While there was no adult female to use as a control in trials where the resident colony lacked an adult female, in four trials a large penultimate female was used as a control instead

adult females, aggression may be more strongly influenced by a spider's reproductive status than by its nestmate status. Adult females may be eliminating other adult and subadult females that might usurp the retreat and breed regardless of kinship, rather than excluding aliens from a limited resource. Our data, however, cannot distinguish between these two hypotheses. In either case, the prevalence of aggression among adult females, suggests that there must be some cost for multiple adult females to cohabit within a single retreat.

In our studies, aggression levels were relatively low (in 23% of trials) compared to the extreme aggression originally reported by Rowell and Avilés (1995). Rowell and Avilés (1995) included data from 12 introductions, 11 of which were with unrelated spiders. Ten of these 11 introductions resulted in the death of the alien or in the alien killing colony members. Rowell and Avilés (1995) also reported three instances when juveniles or subadults attacked or killed other spiders. While immature spiders were present in most trials, we recorded only two trials with attacks by subadults or juveniles. Part of this discrepancy is related to differences in experimental conditions, as our introductions were done with as little disturbance as possible to the introduced animals or resident colony. Colonies in our study were provided with acceptable retreats and were well fed in comparison to those in the earlier study (D. Rowell, pers. comm.).

Another reason may be the decreased aggression seen in the laboratory raised spiders used in this study.

Our results are more consistent with those of Beavis et al. (2007), who found that in 11 of 34 trials *D. cancerides* juveniles cannibalized non-kin (all did so within 24 h). In the remaining trials, spiders starved before cannibalizing kin or non-kin (Beavis et al., 2007). Our finding that older spiders are more likely to experience aggression was also consistent with the Beavis et al. (2007) study, which showed that females did not attack alien juveniles unless they reached a certain size (a carapace width in excess of 6 mm, which is equivalent to the seventh instar, our unpubl. data). While Beavis et al. (2007) confirm our results that most *D. cancerides* refrain from aggression against aliens, their rates of aggression by juveniles are still much greater than suggested by the two instances of aggression by juveniles or subadults reported here. The discrepancy may be again due to differences in husbandry as their animals were housed in very small containers, and our results show that high spider density promotes aggression. Our inability to detect aggression preferentially directed toward aliens may therefore have two causes: one, the rarity of aggression by immature spiders effectively limited the opportunity to observe such preferential aggression. Two, most aggression was between adult females, and we could not determine whether this aggression was due to the elimination of aliens or reproductive competitors.

Our rate of aggression is also low compared to Evans (1999) and Bilde and Lubin (2001), who also examined preferential cannibalism of non-kin spiders in a social context, though direct comparisons cannot be made because we did not starve our spiders or record aggression weeks after introduction. Instead we were concerned with short-term reactions and the exclusion of aliens from the retreat by well fed animals. Our aggression rates might be considerably higher had we included trials with starved spiders.

Spiders in wild colonies were strikingly more aggressive than those in laboratory colonies. This difference may be due to laboratory conditions that blur nestmate recognition cues. Spiders from colonies in the field live under the bark of different tree species in different habitats, eat a wide variety of arthropod prey, and each retreat has been used for a variable number of generations resulting in a build up of prey remains at the base of the colony. In contrast, animals raised in the lab are housed in similar glass terraria and given the same substrate and food. Numerous studies have shown that the recognition cues of social insects are partly derived from the nest, and standardized nest material may make recognition more difficult (Obin, 1986; Singer and Espelie, 1992; Breed et al., 1995; but see Ross and Gamboa, 1981). If all spiders, regardless of colony, share nest-derived recognition cues, perhaps spiders face greater uncertainty (a greater overlap between alien and colony member cues) that would temper their interactions. Laboratory conditions may also disrupt aspects of the spiders' life cycle that regulate aggression. For example, aggression could be dispersal mediated, so that spiders that are unable to disperse reduce aggression to avoid killing siblings. In the laboratory, spiders were given few dispersal opportunities. The higher levels of aggression in wild animals indicate that spiders in the field are likely to behave more aggressively than reported here. However, because spiders live under bark, detailed behavioral observations in the field have been difficult. Fully describing the role of nestmate recognition in intercolony contacts in *D. cancerides* awaits rigorous field studies, through the use of nest boxes or other manipulations of the retreat to allow non-intrusive observations.

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