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Aggregations of unrelated *Apis florea* colonies*

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Abstract – Intensive surveys of an area of woodland in Phitsanulok province, Thailand, revealed 15 colonies of *Apis florea*. The colonies had a highly aggregated spatial distribution (Standardized Morisita's Index of Dispersion = 0.59). Microsatellite analysis based on 5 loci showed that no colonies were related as mother-daughter, suggesting that unrelated colonies tend to nest near existing colonies.

aggregation / *Apis florea* / Thailand /dwarf honey bee / colony relatedness

1. INTRODUCTION

Animals and plants often have a clumped spatial distribution, which is usually assumed to arise from heterogeneity in the environment, with the distribution tracking favourable sites (Forman and Gordon, 1987). Even when no environmental heterogeneity is discernable, it is often assumed that clumped distributions arise from cryptic environmental heterogeneity. More recently, it has been recognized that spatial patterns can arise through self-organized interactions (Ball, 1999), for example between density-dependent predation and population growth (Maron and Harison, 1997; Vandermeer et al., 2008) or simple attraction between individuals of the same species (Sumpter, 2006).

Four species of honey bee are often found to nest in aggregations. The giant honey bees, *A. dorsata* Fabricius and *A. laboriosa* Smith, are the most extreme in this regard, often forming massive aggregations of nests, with

well over 100 colonies sharing a single large tree or cliff face (Underwood, 1986, 1990; Oldroyd et al., 2000; Paar et al., 2004; Oldroyd and Wongsiri, 2006). *A. mellifera* L. forms loose aggregations, in which up to 10 colonies may be found in an area as small as 1 hectare (Oldroyd et al., 1995; McNally and Schneider, 1996; Baum et al., 2005). The two dwarf honey bee species *A. florea* Fabricius and *A. andreniformis* Smith also form loose aggregations of nests (Rinderer et al., 2002). This is despite the fact that *A. florea* and *A. andreniformis* are both open-nesting species, and so a heterogeneous distribution of suitable nest sites seems an unlikely cause of nest aggregations in the dwarf bees (Koeniger, 1976). Baum et al. (2005) suggested that patchy distributions of suitable nesting cavities may contribute to the phenomenon of nest aggregation in *A. mellifera*. Nonetheless, aggregations of *A. mellifera* have also been observed in environments where the number of available cavities is extremely large (Oldroyd et al., 1995).

The adaptive significance (if any) of nest aggregation in *Apis* remains unclear. Nesting in aggregations is likely to be associated with at least some costs including: (i) increased

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potential for the transmission of parasites and pathogens between nests; (ii) local depletion of floral resources leading to nutritional competition among colonies and (iii) increased potential for detection by predators (Oldroyd et al., 2000). However, the fact that nest aggregations occur in at least 4 of the 9 extant honey bee species suggests that there are likely to be adaptive benefits of aggregation that outweigh these potential costs. Briefly, the suggested benefits are (i) swarms might seek to join aggregations because of the potential for cooperative nest defense among colonies (Seeley et al., 1982). This seems unlikely because at least in *A. dorsata*, adjacent colonies rarely join defensive swarms when a colony is attacked by a mammalian or avian predator (Kastberger and Sharma, 2000); (ii) swarms might seek to join aggregations as a means to improving the mating success of offspring queens and drones (Oldroyd et al., 2000). Honey bees mate on the wing under potentially hazardous circumstances. Thus the nearby presence of large numbers of colonies may enhance fitness by providing large numbers of unrelated males so that inbreeding is avoided and queens can mate expeditiously (Oldroyd et al., 2000); (iii) swarms may be attracted by the presence of other successful colonies, as they indicate that an area is resource rich (Oldroyd et al., 1995, 2000).

There may also be non-adaptive causes of nest aggregations including: (i) a heterogeneous distribution of suitable nesting sites or hazards like insecticide application; (ii) endogenous cluster formation caused by interactions between factors unrelated to the physical environment (Czárán and Bartha, 1992; Ball, 1999); (iii) migrating swarms tending to aggregate at the same place because they follow the same behavioural rules during migration (Oldroyd and Wongsiri, 2006).

The red dwarf honey bee, *Apis florea*, of south east Asia and India builds a single comb suspended from a twig or small branch, within which it stores honey and pollen and raises brood (Wongsiri et al., 1997; Oldroyd and Wongsiri, 2006). The species is highly migratory, with colonies moving into areas where floral resources are abundant, and leaving when resources decline (Pandey,

1974; Sheikh and Chetry, 2000; Oldroyd and Wongsiri, 2006). Little is known about reproductive swarming in this species. Akkratanakul (1977) observed one reproductive swarm depart the parent nest and settle about 20 m away, but did not record whether this was a temporary cluster or if the swarm subsequently left to build a comb elsewhere.

In this study, we examined the natural distribution of and relatedness of nests of *A. florea*, in a heavily forested area of Phitsanulok province in lower northern Thailand. The area studied was homogenous, and there was no pesticide usage, so environmental factors are unlikely to have caused aggregation. In this respect our study contrasts with that of Rinderer et al. (2002) who found evidence for aggregation of nests of the dwarf honey bees in an area where horticultural plantings created a heterogeneous environment. We also provide the first investigation of the relatedness of nests within and between aggregations of *A. florea* using microsatellite genetic markers. Thus we were able to determine if clusters arise via short-distance dispersal of reproductive swarms.

2. MATERIALS AND METHODS

The survey was conducted in an area of Muang District, Phitsanulok province, Thailand. The surveyed area comprised numerous shrubs and trees and some buildings. We surveyed the area intensively for a period of 4 months from June–September 2004. This period is during the rainy season when *A. florea* populations are low. Each tree and other possible nest sites were observed by at least two people at a time.

When nests were located, their precise location was determined with the aid of a Global Positioning System receiver (Garmin®, Garmin Taiwan). The positions of nest were then mapped using the ArcView GIS 3.2 program (ESRI). We overlaid the colony locations on this map with quadrats of size 400 × 400 m, and used the number of colonies per quadrat to calculate the standardized Morisita's index of dispersion (Krebs, 1989). This index provides one of the best estimates of biological dispersion because it is independent of density and population size (Krebs, 1989).

From each colony, we collected 40 adult workers. DNA was extracted from the thorax of each bee

using Chelex[®] resin (Walsh et al., 1991). Diluted DNAs (1:4) were amplified in a thermocycler using microsatellite loci isolated from *A. mellifera* (A8, A76, A88, A107), and *Bombus terrestris* (B124) (Estoup et al., 1993), but known to amplify cleanly in *A. florea* (Palmer and Oldroyd, 2001). PCR products were then electrophoresed in 12% denaturing polyacrylamide gels. After electrophoresis, the amplified products were visualized using silver staining (Bassam et al., 1991).

We determined the expected heterozygosity for all loci from the observed allele frequencies. At each locus for each colony, we inferred the genotype of the queen heading the colony from the distribution of worker alleles (Estoup et al., 1994; Oldroyd et al., 1996). We then determined which if any colonies could be related as mother and daughter from the genotypes of the inferred queen mother pairs. A mother and daughter queen must share at least one allele at all loci.

3. RESULTS

We found 3 aggregations of *A. florea* within the study area, of sizes 6, 5 and 4 colonies (Fig. 1). For this distribution the Standardized Morisita's Index of Dispersion is 0.59 indicating that the distribution of the *A. florea* nests was significantly aggregated (Krebs, 1989). In aggregation 1, there were two colonies nesting in the same tree (colonies 4 and 5).

Although nests were highly aggregated at the broader scale, they were not highly aggregated at the scale of aggregation. The closest distance between nests within aggregation 1 was 1.7 m; 22.9 m in aggregation 2 and 80 m in aggregation 3 (Fig. 1). The average distance between nests within aggregation 1 was 188.6 m, 80.0 m in aggregation 2 and 135.2 m in aggregation 3. The nearest distance between nests in aggregation 1 and aggregation 2 was 2274.3 m, aggregation 2 and aggregation 3 was 1565.7 m and 937.1 m between aggregation 1 and aggregation 3 (Fig. 1). These densities are similar to those seen in aggregations of *A. mellifera* (Oldroyd et al., 1995; McNally and Schneider, 1996; Baum et al., 2005).

The inferred genotypes of each queen are shown in Table I. No pairs of colonies were related as mother-daughter because no pairs of inferred queen genotypes shared at least one

allele at all loci. We cannot exclude the possibility that some colonies were headed by half-sister queens, but we did not analyze sufficient loci to test this hypothesis statistically.

4. DISCUSSION

Our results show that the previously-reported tendency for *A. florea* nests to have a clumped spatial distribution (Rinderer et al., 2002) did not arise as a consequence of heterogeneity in suitable nesting sites. Our study, conducted in habitat with a uniform distribution of trees suitable for nesting, revealed a clumped distribution of nests, which if anything was even more striking than that seen in the Rinderer (2002) study.

Our study also shows that like *A. dorsata* (Oldroyd et al., 2000; Paar et al., 2004) and *A. mellifera* (Oldroyd et al., 1995) aggregations of *A. florea* do not arise from the clustering of daughter colonies around a single founding colony. No colonies in our study were related as mother-daughter, for no pairs of inferred queen genotypes shared one allele at all loci studied. Rather, our study suggests that unrelated swarms, either migratory or reproductive, preferentially nest near existing nests. An aggregated spatial distribution of *A. florea* colonies, a species whose nest site requirements are not exacting, strongly suggests that colonies preferentially nest near existing colonies, and implies that aggregations in other species also arise from preferential nesting near existing colonies. The absence of daughter colonies may suggest that reproductive swarms leave the immediate vicinity of their parent colony and disperse, possibly to another aggregation.

It remains unclear as to why honey-bee colonies should have an aggregated spatial distribution. Mutual nest defense appears unlikely given that the mean distance to nearest neighbor was 65.81 m. This distance seems too large to permit any kind of defensive interaction between nests. Vandermeer et al. (2008) showed that ant colonies can acquire an aggregated spatial distribution via an interaction of density dependent predation and short-distance dispersal of offspring nests from the

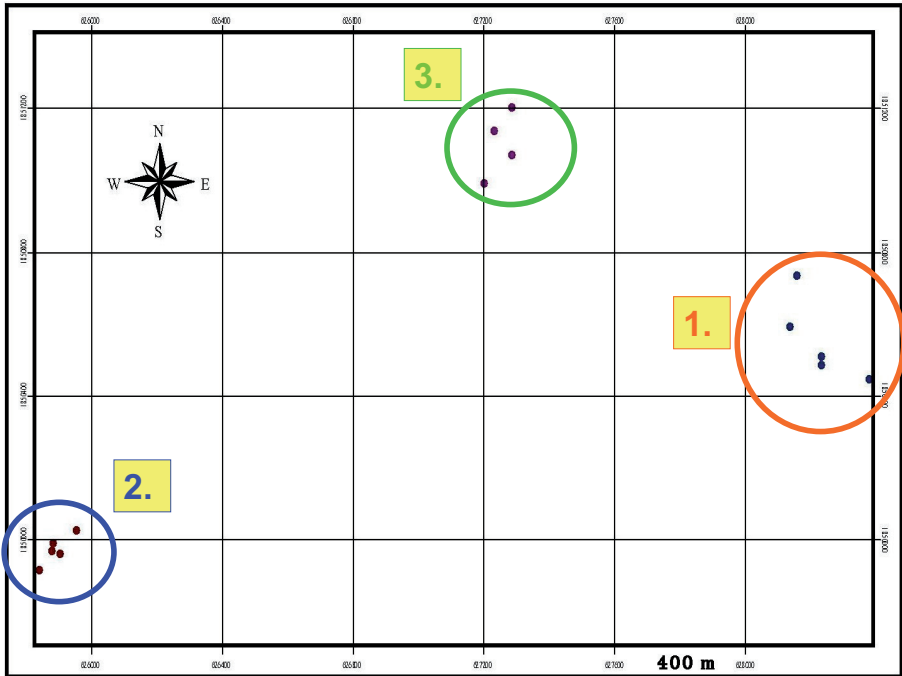


Figure 1. Distribution of *A. florea* nests in Phitsanulok province Thailand during 2004. Squares are 400 m quadrats.

Table I. Inferred queen genotypes of 15 *A. florea* colonies within 3 aggregations in Phitsanulok, Thailand, at 5 microsatellite loci and expected heterozygosities (H_E) at those loci.

Population	Colony	Queen alleles				
		Locus A8	Locus A76	Locus A88	Locus A107	Locus B124
1	1	150/154	194/196	139/141	106/110	193/195
1	2	154/156	199/201	141/145	106/110	187/191
1	3	154/156	204/208	146/148	104/106	189/191
1	4	157/159	200/204	143/145	106/110	191/193
1	5	154/156	198/202	143/145	100/108	189/191
1	6	154/156	205/207	137/141	102/104	186/190
2	7	154/156	186/190	139/143	102/104	186/190
2	8	150/156	195/197	140/142	102/106	184/186
2	9	150/154	197/201	135/139	102/106	182/186
2	10	154/156	193/195	139/141	102/108	180/186
2	11	154/156	183/185	142/144	102/106	182/184
3	12	153/157	193/195	133/137	104/106	192/194
3	13	155/157	185/187	132/132	104/106	193/195
3	14	158/160	191/193	132/134	104/106	191/193
3	15	153/155	187/191	139/141	102/104	189/191
H_E		0.86	0.92	0.89	0.79	0.90

parent nest. Our genetic analysis shows that clusters are not comprised of a mother nest surrounded by daughter nests. Thus spatial aggregation observed in honey bees is almost certainly does not arise via self-organizing factors, but by swarms being attracted to the neighborhood of existing nests. Why swarms should be so attracted remains a matter of conjecture, but increased mating opportunities and inbreeding avoidance remain an interesting hypothesis.

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Agrégations de colonies d'*Apis florea* non apparentées.

Apis florea / agrégation / abeille naine / Thaïlande / parenté

Zusammenfassung – Nestansammlungen von nicht verwandten *Apis florea* Kolonien. Bei vier der Honigbienenarten werden Nester oft in gehäuften räumlichen Verteilungen vorgefunden. So können zum Beispiel bei den Riesenhonigbienen *Apis dorsata* und *A. laboriosa* mehr als 100 Kolonien an einem einzelnen Baum oder Felsüberhang nisten. Auch die rote Zwerghonigbiene, *A. florea*, weist eine gehäufte räumliche Verteilung der Nester auf. Eine offensichtliche Erklärung von Nestansammlungen ist eine ungleichmäßige Verteilung geeigneter Nistplätze. Dies ist allerdings bei *A. florea*, die ihre Nester an kleinen Baumästen anlegt, eine wenig wahrscheinliche Erklärung. Es ist daher schwer zu sehen, dass hier ein Mangel an geeigneten Nistplätzen zu einer gehäuften räumlichen Verteilung führen sollte.

Für die Entstehung von Nestansammlungen bei Honigbienen gibt es mindestens vier plausible Hypothesen: (i) Schwärme könnten sich wegen der Möglichkeit kooperativer Nestverteidigung Ansammlungen anschließen; (ii) Schwärme könnten sich zur Verbesserung des Paarungserfolges von Königinnen und Drohnen Ansammlungen anschließen; (iii) Schwärme könnten von der Anwesenheit anderer erfolgreicher Kolonien als Anzeiger einer ressourcenreichen Umgebung angezogen werden; (iv) Ansammlungen könnten durch sehr kurze Wegstrecken der reproduktiven Schwärme hervorgerufen werden, oder dadurch, dass wandernde

Schwärme während ihrer Wanderung den gleichen Verhaltensregeln folgen.

Wir untersuchten die natürliche Verteilung und die Verwandtschaftsbeziehungen von Nestern der roten Zwerghonigbiene *A. florea* in der Phitsanulok Provinz im tieferen nördlichen Thailand. Die Verteilung der von uns gefundenen 15 Völker war stark gehäuft (Abb. 1: Morisita's Verteilungsindex > 0,5). Die Untersuchung von Arbeiterinnen dieser Nester anhand vom Mikrosatelliten zeigte, dass keine der Nester eine Mutter-Tochter Verwandtschaft aufwies. Daher konnten diese Ansammlungen nicht auf Grund von geringer Ausbreitung der Nachkommenskolonien vom Ursprungsneue entstanden sein. Eher legen unsere Ergebnisse nahe, dass Schwärme von Gebieten angezogen werden, die Nester enthalten.

Aggregation / *Apis florea* / Thailand / Zwerghonigbienen / Kolonieverwandtschaft

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