Distribution, Ecology and Behavior of *Anochetus kempfi* (Hymenoptera: Formicidae) and Description of the Sexual Forms

by

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ABSTRACT

The ponerine, *Anochetus kempfi* Brown, is a cryptic nocturnal ant, widely distributed in Puerto Rico and the Virgin Islands. It is found in various habitats ranging from dry forest to rain forest. Males and the ergatoid queens are here described and illustrated for the first time. Mature colonies contain about 100 workers and may include several queens. We have observed males flying every month except March. In artificial nests we have observed that workers execute excess queens and males which do not depart from the nest within a short time following eclosion. These executions appear to be mechanisms to enforce outbreeding in the case of males, or to force emigration of excess queens to establish new colonies. Few queens are produced and this could be related to local resource competition. Permanent egg carrying by workers occurs in this species, a behavior not previously observed for any ant species.

Key words: ants, behavior, Puerto Rico, queen execution, tropics.

INTRODUCTION

There are only 13 species of the ant subfamily Ponerinae in Puerto Rico, mostly of secretive habits. Members of this subfamily exhibit partially claustral colony founding (Peeters 1993, Dejean and Dejean 1998). Ponerines are relatively large ants and the queen-worker dimorphism is weak (Villet *et al.* 1991). This weak dimorphism seems to be one of the reasons why queens could not raise a first generation of workers based only on the founding queen's reserves (Dejean & Dejean 1998).

In the genus *Anochetus* there are two species in Puerto Rico: *A. mayri* Emery and *A. kempfi* Brown. The latter species was described only from workers (Brown 1978); the males and queens were unknown and have remained undescribed. Males found associated with the female castes

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are known for a minority of the species in the genus (Brown 1978). The paucity of information on the ecology and behavior of *Anochetus* species is legendary. *Anochetus* is considered the sister genus of *Odontomachus* and both genera have a fast mandible strike known as a trap-jaw mechanism (Gronenerg and Ehmer 1996). *Anochetus kempfi* is a predatory nocturnal species with fast moving workers that hide as soon as they detect artificial light. We have found that the cephalic extracts of *A. kempfi* workers contains 2-5-dimethyl-3-isoamylpyrazine and 3-methyl-4-phenylpyrrole, probably these heterocycles are mandibular gland products with a pheromonal role. (Jones *et al.* 1999, Morgan *et al.* 1999). In this paper we provide information on the distribution, natural history, and behavior of this species. Since the sexual forms of this species have not been previously described, we take this opportunity to do so.

MATERIALS AND METHODS

Specimens of *A. kempfi* were collected by searching for foraging workers, as well as a variety of other methods, including pitfall traps, baiting with tuna fish, searching for nests under logs, rocks, coconut seeds and other objects, and by light traps and Malaise traps (flying males only). Some nests were located by following foraging workers returning to their nests.

Colonies were collected at Cabezas de San Juan (Fajardo, Puerto Rico) and established in artificial nests made of plaster of Paris placed in plastic tubs. The chambers of the artificial nests were covered with glass to facilitate observations (Hölldobler and Wilson 1990). Colonies were fed worker termites (*Cryptotermes brevis* Walker and *Nasutitermes costalis* Holmgren), fruit fly larvae (*Drosophila* spp.), mealworms (*Tenebrio* sp.) and diluted honey. Colonies were fed daily and left exposed to dim light from 5-10 minutes to acclimatize these nocturnal ants to light conditions. Behavioral observations were made using a red light, and behavioral sequences were recorded under both infrared, and dim white light with a video cam.

The first observation colony was set up on April 11, 1998 with 66 workers, 10 pupae, 3 queens and 3 males. Three more colonies were established in artificial nests on June 21, 1998. These nests were composed of the following: #2, 40 workers, 20 pupae, 3 queens, 4 males; #3, 53 workers, 19 pupae, 2 queens, 1 male; #4, 42 workers, 6 pupae, 2 males. The queenless colony (No. 4), was set up to determine whether or not workers would attempt reproduction in the absence of a queen.

RESULTS

Distribution

Brown (1978) described this species from seven worker specimens, all from Puerto Rico: six from Culebra Island and one from Cataño, near San Juan. The Culebra specimens had been erroneously recorded by Wheeler (1908) as *A. testaceus* Emery; these had been collected from "colonies nesting under stones in the shade of trees along the dry arroyos on the higher part of the island (Monte Resaca)."

In addition to the two records cited by Brown, we have material from the following Puerto Rican localities. Municipalities: Arecibo, Guánica Forest (Guánica), Loíza, Luquillo, Río Piedras, San Lorenzo, Susúa Forest (Yauco), Trujillo Alto. Small islands and keys: Cabeza de Perro, Cayo Ratones (near Fajardo), and Culebra. We have also collected this species in the British Virgin Islands, (Guana I.) and the U. S. Virgin Islands (Inner Brass, near St. Thomas).

Anochetus kempfi inhabits dry forest (Guánica; Culebra; Cayo Lobos; Guana I.; Inner Brass), rain forest (Bisley, El Yunque), moist forest (Susúa), sandy beach (Loíza), pasture land (San Lorenzo; Luquillo), and urban woodland (Trujillo Alto). Nests are difficult to locate and can be found in soil, in decomposing wood, under coconut seeds (our observations), and beneath stones (Wheeler 1908).

Ecology and behavior

In natural nests there are from two to four queens per nest (N = 5). The queens (including newly eclosed callows) hold the gaster elevated somewhat above the plane of the mesosoma, in contrast to workers in which the gaster is held at the same level as the mesosoma. Queens are largely ignored by worker ants and do not seem to receive special attention; workers preferentially care for larvae and pupae. The queens feed directly on prey brought into the nest by returning foragers.

In artificial nests, queens remained close together and did not interact aggressively during the first two or three weeks. Thereafter something triggers a different behavior and "extra" queens were attacked by the workers. While workers normally exhibit minimal cooperation, such attacks may involve 16 or more attacking a queen. Sometimes, too, another queen may participate in the execution of one of these supernumerary queens. Such attacks may last several days. The attacked queens are bitten. and stung by the workers and are eventually ejected from the nest. Some of the ejected queens had been observed to be producing eggs.

On November 4, 1998 two virgin queens were produced in the first queen-right nest. As noted above, these, too, hold the gaster in an elevated position. They were observed feeding on termites and remaining near the old queen, possibly reflecting some queen microhabitat preferences. Two callow workers were attacking the old queen the next day. Although we did not observe aggression against the new virgin queens, both were found dead four days after eclosion. Four virgin queens eclosed in the third queen-right nest on July 26, 1999. This nest was moved to a bigger plastic tray and two additional artificial nests were laid 40cm from the occupied nest to see if the colony would split and occupy the new nesting sites. Seven days later, the workers attacked one of the virgin queens and the queen was ejected from the nest. Twenty-one days later the three remaining virgin queens and the old queen were licking each other's gasters. After the death of one of the virgin queens, we did not observe worker aggression against any of the remaining four queens for a period of seven months. Also, there was no aggression among the queens, which remained near each other in the nest. The workers occupied the two new nesting sites and the queens, on three occasions, moved to one of the new nests, but after two or three days in the new nest they returned to the old nest.

Males in field nests (N = 8), from 0 to 8: January 12, 1998 (0, 8, 0), April 11 1998 (3), June 21, 1998 (4, 1, 2), July 22, 1995 (1). A total of 38 males were produced in the artificial queen-right nests during a period of seventeen months. Males were collected in light traps at Guánica dry forest every month except March.

Males eclose fully pigmented and die or are killed within two weeks if they do not abandon the artificial nest; males that are killed are sometimes eaten by the workers. Some males were killed four days after eclosion. Workers have not been observed to feed the males and males apparently are unable to feed themselves.

With their reduced, apparently weak mandibles, males appear to be incapable of freeing themselves from the pupal cocoon. Their eclosion may be assisted by up to three workers. Once emerged, they are cleaned by up to five workers; males are quiet during this cleaning and may be carried from one chamber to another by the workers.

There are from 44 to 97 workers per nest (N = 4). Wheeler (1908) found that in the colonies he observed on Culebra, the numbers of workers in a nest ranged from about 30 to 100. Workers keep their forelegs in the air while stinging prey. Prey is captured with the mandibles and stung at once; although prey may be stung several times, usually a single sting is sufficient to induce paralysis. Workers can jump by "mandibular clicking" (Hermann and Blum 1981).

Workers do not seem to employ alarm pheromones nor do they recruit. Worker cooperation is minimal; at most two workers may work together to bring a prey item to the nest. Prey arrival does not cause excitement within the colony, as is so commonly true with other ant species. It sometimes happens that a large caterpillar may be attacked by several workers at the same time, but apparently each encountered the prey independently. We found worker trophallaxis to be uncommon. Groups of three to five workers are commonly seen feeding on the same item. When feeding, workers preferentially grasp the prey in the forelegs and held the prey off the ground. Although the worker mandibles are very useful for capturing prey, they are not very useful for cutting prey into pieces. The mandibles have to be opened widely so that the central inner mouth parts can reach the food, while the food is held by the forelegs or by the mandibles of another worker.

Mature workers in the queenless nest never laid eggs. Workers lived for a maximum of 259 days after their capture and were "restless" when exposed to light. The presence of immatures in a nest tends to have a calming effect on workers.

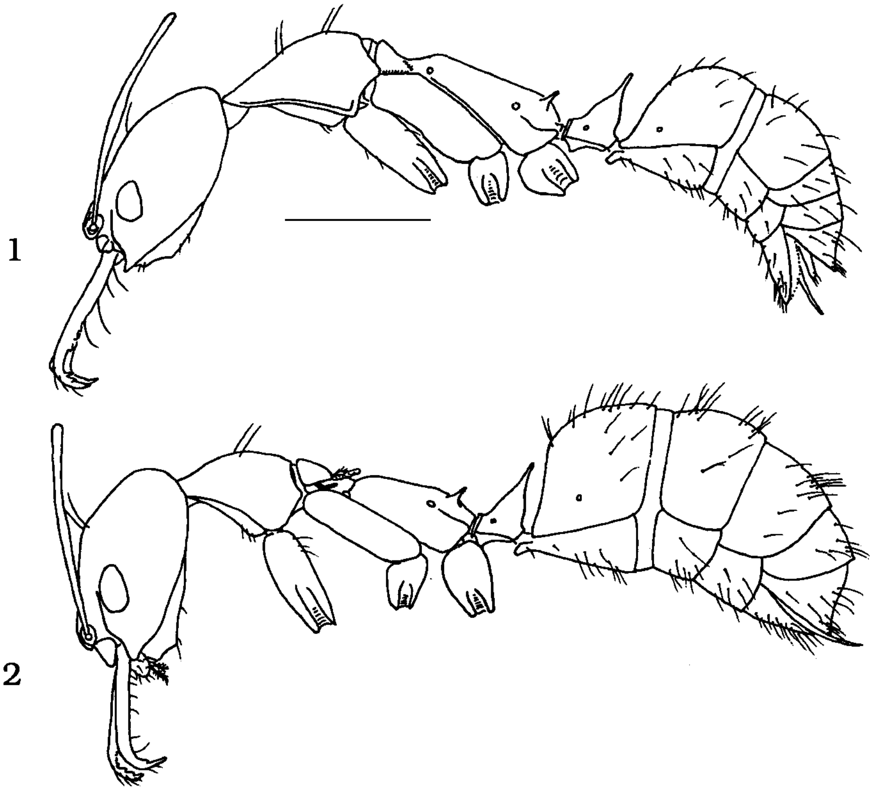
Eggs are not allowed to lie on the floor of nest chambers; they are held, usually in clusters or packets, by the workers. Up to five workers may be seen holding egg packets in their mandibles; this duty is sometimes assumed by newly eclosed, callow workers. One of the laboratory queens was seen to produce an egg that was picked up by one worker and put on an egg cluster held by another worker. Some workers held the eggs in their mandibles for days and are very reluctant to transfer the egg cluster to another worker. Sometimes the transfer of the egg cluster from one worker to another is accomplished by force.

Larvae and pupae lie on the floor of nest chambers. Prey is put on the mouthparts of the larvae, which feed directly on the food item. While some apparent trophallaxis was observed, it is uncommon. Larvae need loose soil to pupate. Following pupation, workers clean the new pupal cocoon of soil particles; up to three have been seen cleaning a single cocoon.

Description of sexual forms

Queens (Figs. 2, 3): ergatoid, as predicted by Brown (1978). Measurements (mm): head width 1.33-1.37; head length 1.57-1.60; mandible length 1.03-1.07; scape length 1.60-1.63; Weber's length 2.43-2.47; total length 6.97-7.22. Ratios: cephalic index 85; mandibular index 66-67; scape index 102. N = 2.

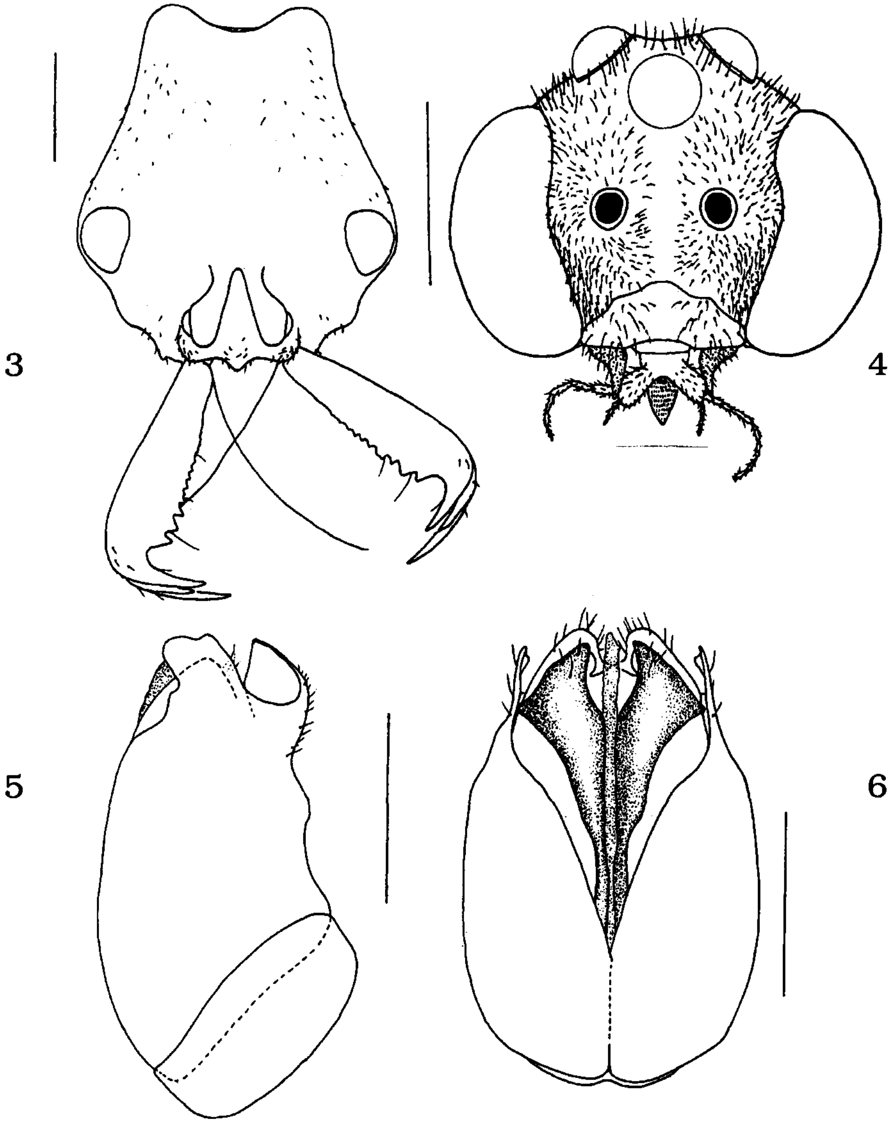
Queens (Fig. 2) are slightly smaller than their workers (Fig. 1) and similar in color, varying from light to dark ferruginous yellow; appendages are paler except that tarsi are contrastingly brownish. Being



Figs. 1-2. *Anochetus kempfi*, profile of head, meso- and metasoma of: 1, worker; 2, queen. Scale line = 1.00mm.

ergatoid, the queens are similar to their workers in appearance; ocelli are not present; the only obvious difference is the presence of distinctly demarcated meso- and metanotal sutures across the mesosomal dorsum (Fig. 2); one of the two queens examined has vestigial wings that are obviously non-functional; the gaster is more voluminous than that of workers, even in an unmated callow queen.

Males (Figs. 4-6): Measurements (mm): Head width 1.00-1.10; head length 0.80-0.83; Weber's length 1.8-2.0; total length 4.8-5.3; wing length 3.9-4.5. Cephalic index 125-133. N = 5 (including apparent largest and smallest individuals available). Mandibles vestigial, as usual in *Anochetus*, edentate. Eyes prominent, 1.4-1.6 x longer than wide in profile and 1.0-1.2 x longer than upper interocular distance; in frontal view, upper interocular distance 1.4-1.6 x lower interocular distance. Flagellar segments long, third antennal segment about 3 x as



Figs. 3-6. *Anochetus kempfi*: 3, frontal view of queen head; 4, frontal view of male head; 5, profile of male genital capsule; 6, dorsal view of male genital capsule. Scale line = 0.50mm.

long as wide and about 3 x as long as first segment (scape). Vertex strongly convex; ocelli large and ocellocular distance distinctly less than diameter of anterior ocellus. Integument shiny between minute piligerous punctures.

Mesosoma shiny between obscure fine piligerous punctures; scutellum strongly convex; propodeum, in profile, sloping and mostly flat, posterior declivity weakly differentiated.

In profile, anterior face of petiole node evenly sloping, summit broadly rounded into short posterior declivity; anteroventral process well developed, its apex obliquely acute.

Metasomal terga shiny, punctures slightly better defined than elsewhere on body. Subgenital plate almost twice as long as broad, sides subparallel or slightly convergent apicad, posterior margin shallowly concave to nearly transverse. Genitalia as illustrated (Figs. 5, 6).

Color yellowish brown to light brown; mandibles and legs yellowish; first two antennal segments yellow, 3-13 brown; variable areas of lower face, gena, side of mesosoma and apical margins of metasomal segments also yellowish.

Head and body with abundant short, suberect yellowish hairs and scattered conspicuously longer suberect yellowish hairs; on metasoma longer hairs abundant on sterna and progressively more abundant on successive terga.

DISCUSSION

Several explanations have been suggested to explain queen execution by workers. Keller *et al.* (1989) postulate that queen execution is a mechanism to reduce queen inhibition on the differentiation of sexuals in the polygynous Argentine ant, *Linepithema humile* (Mayr). Later, Keller and Ross (1993, 1998) conjecture that execution of reproductively superior queens may represent a mechanism to maintain polygyny in colonies of the fire ant, *Solenopsis wagneri* Santschi (as *S. invicta* Buren).

Among African driver ants (*Dorylus* spp.), once the functional queens left the nest, daughter colony workers kill all but one of the virgin queens (Gotwald 1995). In this case, regicide is assumed to be a mechanism to establish monogyny in these army ants.

In the case of *A. kempfi*, we think that new colonies are normally established by fission; probably the (virgin?) queen and some workers may migrate, leaving the old queen with a colony remnant. Thus, it may be that queen execution by workers in the artificial nests may be a response to unnatural conditions that may prevent the normal process of emigration of a portion of the mature colony. Possibly, in nature, multiple queens coexist in one nest by dispersing through several galleries and chambers. Then, if excess queens cannot depart to found new nests or if they are too close to one another, they are attacked, such attacks sometimes resulting in death of the queens. The attacks on queens or coexistence of *Anochetus kempfi* queens did not follow any consistent pattern in terms of kinship. We doubt that queen execution is a common behavior in field nests because this species produces only a small number of queens, which cannot fly.

Unlike queen execution in *L. humile*, which occurs during the first week after establishment of the colony in the laboratory (Keller *et al.* 1989), in *A. kempfi* queen execution took place two to three weeks later. Keller and Ross (1993) studied queen execution in *S. wagneri* during the first week after queen introductions. However, no generality may be drawn from their observations; we have observed that such executions may take place later, in the case of *A. kempfi*. Also Leal and Olivera (1995) found that workers attacked and killed one of the queens in a pleometrotic association of *Pachycondyla marginata* five months after collection.

In *A. kempfi* we observed attacks on males and we believe this is a mechanism to force males to depart in search of new nests with non-sibling virgin queens. Surprisingly, some of the executed males, but not queens, were eaten by the workers. In *Cardiocondyla nuda* (Myrmicinae), Heinze *et al.* (1993) found that ergatoid males attack male callows and in one occasion a callow was cut in pieces and fed to the larvae, but we did not observe workers of *A. kempfi* feeding male pieces to the larvae. More males than females were produced in the artificial nests. Since many decads of males have been collected at a single Malaise trap on a single night, this is probably a normal situation.

Although Villet (1991) was able to establish semiclaustral haplometrosis nests of the ergatoid ponerine *Plectroctena mandibularis* in captivity, the apparent absence of wing muscles in ergatoid queens seems to make difficult the establishment of a colony without help from workers (Trunzer *et al.* 1998). In ponerines with wing muscles and pleometrotic associations even some queens must forage (Trunzer *et al.* 1998). Because queens in *A. kempfi* are ergatoid and do not fly, we expect colony budding or colony fission to be the mechanism by which new colonies are established. In colony budding (or fission), daughter colonies remain near each other and the parent colony, resulting in local resource competition between relatives (Bourke and Franks 1995). When competing relatives are females, local resource competition promotes male-biased investment and male-biased numerical sex ratios when males are cheaper to produce than females as is usually the case in ants. Although ergatoid queens are cheaper to produce compared to non-ergatoid queens, because they do not need resources for the production of wing muscles and fat reserves, the cost of the workers which depart with the queen are considered part of the investment in a female. Thus, local resource competition could be a possible reason for the high male production in *A. kempfi*.

An alternative explanation is that females are exposed to fewer risks since they do not take mating flights. Queens are wingless in *A. kempfi* and males seek out nests with virgin queens with which to mate. Although we have not witnessed mating in this species, it probably takes place within or near the nest. Because males are more exposed to predators and such other inconveniences as flying to the wrong location, probably only a small minority actually gain access to a new nest. Perhaps this is a reason for the high frequency of male flights in this species. It is difficult to single out one alternative as the sole cause for the bias in numerical sex ratios. Perhaps both factors are responsible for the numerical male-biased sex ratio.

We did not find worker egg production in the queenless colony; probably *A. kempfi* workers lack ovaries as in other *Anochetus* species (Villet *et al.* 1991). Thus, trophic eggs are not available and since trophallaxis is rare the queens feed by themselves on the prey retrieved by the workers. This may explain the lack of attention of workers toward the queens. Multiple queens were found in *A. kempfi* nests, but Villet *et al.* (1991) found only one queen per nest in three South African species of *Anochetus* (one of the species has ergatoid queens). We believe this is the first report of multiple queens in *Anochetus*. Recruitment or prey transport by more than two workers was not observed in *A. kempfi*. Similarly recruitment or collective prey retrieval does not occur in the African *A. traegordhi* (Schatz *et al.* 1999).

Permanent egg carrying behavior in workers as reported here is a novelty, not known elsewhere among the ants. We suspect that other such instances will be discovered, at least among other species of *Anochetus*. The adaptive significance is unknown. Perhaps such behavior facilitates nest evacuation in the event of disturbance or emigration when establishing a new satellite colony. Possibly this is a mechanism to avoid egg parasitism or predation, or exposure to soil molds. Another alternative is that egg-carrying behavior in *A. kempfi* workers is a mechanism to avoid oophagy by rival or competing queens. In the ponerine *Pachycondyla villosa* pleometrotic associations occur and queens exhibit oophagy. Eggs are typically eaten by rival queens after they had been laid while the queens held the egg in their mandibles. Eggs are not eaten from the single pile where the eggs are handled by all the queens (Trunzer *et al.* 1998). Although we have not observed queen oophagy in *A. kempfi*, probably rival queens of *A. kempfi* could have more difficulty if they try to eat eggs from a pile held in the mandibles of the workers than from the nest floor.

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