

Effects of Queen Body Parts on the Production of New Sexu- als in the Pharaoh's Ant, *Monomorium pharaonis* (Hymenoptera: Formicidae)

by

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ABSTRACT

The effects of the presence of queen body parts on the production of new sexuals in the Pharaoh's ant, *Monomorium pharaonis* were determined in this study. Whole live queens, whole dead queens and queen's thorax, head and gaster were used respectively in ant colonies consisting of 300-400 workers and 0.5 g of brood. Reproductives started emerging at day 57 after initiation of the experiment. Results showed that replicates with live queens were the slowest to produce sexuals. By day 64, only queenless replicates showed insignificant difference with the queenright colonies. At day 85, replicates with queen's gasters were not significantly different from the queenright and queenless colonies, causing the authors to suggest that suppression pheromones could be stored in the queen's gaster. Production of male alates was irregular throughout the entire 15 weeks of experiment. We hypothesize that a male alate could inseminate more than one virgin queen in its life.

Keywords: *Monomorium pharaonis*, queen, pheromones.

INTRODUCTION

The Pharaoh's ant [*Monomorium pharaonis* (L.)] has emerged to be one of the most important pest species in the world. Its notoriety in bacterial transmission at hospitals through sterilized apparatus makes it an even more burdensome pest. Over the years, many studies on the control of this species have been conducted. This includes the usage of juvenile hormone integrated into baits. Hormones such as this play an important part in caste differentiation in social insects. Although studies were conducted in the 1960s and the 1970s, they provided minimal information needed to fully comprehend the caste differentiation phenomenon. Queens of social insects are known for their ability to suppress the production of new sexuals. They are essentially the principal reproductives, if not exclusive, in eusocial insects. They play an important role in communication through production of numerous

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pheromones to coordinate activities within the colony (Hölldobler & Wilson 1990, Winston & Slessor 1992, Vargo 1998, 1999). Despite its royal importance, progress in understanding insect queen pheromones has been sluggish (Vargo & Hulseley 2000).

The best-studied queen pheromone system in social insects is that of the honey bee, *Apis mellifera* L. (Winston & Slessor 1992). The honey bee queen's mandibular gland produces a complex of five compounds that have several different effects. These effects include formation of a retinue of workers around the queen (Slessor *et al.* 1988), inhibition of construction of queen cells by workers (Winston *et al.* 1989, 1990), swarming suppression (Winston *et al.* 1991), delaying the onset of worker foraging behavior (Pankiw *et al.* 1998) and stimulation of foraging and brood rearing (Higo *et al.* 1992).

In queenless honey bee colonies, workers are found to be able to establish themselves as pseudoqueens, which express many of the characteristics associated with the reproductive physiology of the queen caste (Sakagami 1958). The queen's secretion is attractive to workers and was postulated to constitute a part of the complex queen signal (Katzav-Gozansky *et al.* 2002, as cited by Sole *et al.* 2002).

In ants, queen pheromones of *Solenopsis invicta* received the most attention (Vargo & Hulseley 2000). The poison sac, which houses the poison gland, was demonstrated to be a source of a releaser pheromone that attracts workers to the queen (Vander Meer *et al.* 1980). This poison sac was also found to be the source of queen primer pheromone that inhibits virgin queens from dealation and ovaries' maturation (Fletcher & Blum 1981, Vargo 1997). In addition, the queens produce a pheromone that restrains rearing of male and female reproductives by the workers (Vargo & Fletcher 1986, 1987) as well as a pheromone that causes mutual suppression of egg production among cohabiting queens in multiple-queen colonies (Vargo 1992).

The queen pheromones too play a role in regulating the number of queens in a colony through execution of excess queens by workers (Fletcher & Blum 1983). As rearing reproductives requires a massive amount of energy, social insects typically delay investment in fabricating reproductives until a suitable worker force is established (Klobuchar & Deslippe 2002). Such pheromones are often manipulated to prevent workers from laying eggs capable of developing into males (Bourke 1988), dictate larval feeding at critical periods to distort gyne development in female larvae (Vargo 1998) or simply by stimulating the workers to execute sexual larvae (Edwards & Abraham 1991, Vargo & Passera 1991). Killing of sexual larvae by workers has been well documented in numerous species of ants namely *Myrmica rubra*, *Plagiolepis pygmaea*,

Linepithema humile, *Monomorium pharaonis* and *Solenopsis invicta*. Executions are ignited in colonies of these pest ants when queens are present but not vice versa (Brian 1979, Passera 1969, 1980, Vargo & Fletcher 1986, Edwards & Abraham 1991, Vargo & Passera 1991).

One of the most striking examples of elaborate cooperative behavior comes from social insects. Although it seems perfect from the world outside, yet within the colony itself, conflict entails. In ants, the most significant conflict concerns sex ratio (Passera *et al.* 2001). Social hymenopterans employ the haplodiploid system in sex determination. As a result, workers are generally more related to sisters than brothers whereas the queens are equally related to her sons and daughters (Mehdiabadi *et al.* 2003).

Queens of many social insects produce a wide variety of pheromones that influence behavior, development and physiology of colony members profoundly (Hölldobler & Wilson 1990, Winston & Slessor 1992, Vargo 1998). Queen control is defined by her ability to influence reproductivity of nestmates (Wilson 1971, Fletcher & Ross 1985).

For instance, fire ant queens can indirectly influence nestmate reproduction by producing releaser pheromones that incite workers to eliminate sexual brood (Vargo & Fletcher 1986, 1987, Klobuchar & Deslippe 2002, reviewed in Brent & Vargo 2003). Functional queens are capable of mutual inhibition of egg production. These queens are also able to prevent dealation of virgin alates and inhibit them from becoming reproductively active (Fletcher & Blum 1981).

The queen pheromone may express its property by affecting nestmate development through action on the corpora allata, thus altering the rate of the juvenile hormone release (Barker 1978, 1979, Vargo & Laurel 1994).

Removal of queens in laboratory colonies resulted in appearance of new reproductives. The same phenomenon was observed in a colony containing brood and workers reared in the absence of queen (Peacock *et al.* 1954, 1955). In a nest where fertile queens are present, rearing of new sexual forms was prevented (Edwards 1987).

This study focuses on how queens' presence affects offspring of new reproductives. The origin of the queen pheromone is also studied by manipulating queen's body parts in colonies containing workers and brood.

MATERIALS AND METHODS

Ants used in this study were Pharaoh's ants that have been reared in the Urban Entomology Laboratory since 1995. They were reared in the laboratory using aluminium trays (40.0 x 24.5 x 8.0 cm) with the

inner sides laced with fluon to prevent escape. Food such as cockroach nymphs, tuna fish, hard-boiled egg yolk, honey and sugar water was given *ad libitum*. Water source was ensured to be available at all times using an up-turned polypropylene cup on a petri dish cover. Cotton was used to fill the petri dish cover to provide the moisture to the ants.

Queens were killed at freezing temperature before their body parts were taken. Freshly killed queens were cut accordingly to obtain the head, the thorax and the gaster. Some queens were left intact to be used in the replicates with whole dead queens.

Experimental colonies were set up with each colony consisting 300-400 workers and 0.3 g of mixed-stages brood. Queens' presence was manipulated into different conditions as follow:

- (A) 4 Live queens
- (B) 4 Whole dead queens
- (C) 4 Queen's heads
- (D) 4 Queen's thoraces
- (E) 4 Queen's gasters

Each of the conditions mentioned above was replicated three times and food was provided every other day. Daily observations were made for emergence of new sexuals. After the first sight of new sexuals, surveillance was made on a weekly basis. This was done by taking digital still pictures of each colony. These photos were then used in enumerating the total number of new queens and male sexuals in each experimental colony. Data collected was subjected to One-Way ANOVA and the means were separated using Tukey HSD ($p < 0.05$).

RESULTS AND DISCUSSION

We first observed new female reproductives emerging after 57 days from the initiation of the experiment. Three weeks later, queenless replicates were the only ones insignificantly different from the queenright colonies. At week 6, replicates with queen's gasters were not significantly different from the queenless and queenright colonies (Fig. 1). This shows that the production of new female reproductives may have been suppressed by the presence of queen's gasters.

Results also showed that queen body parts enhanced the making of female reproductives. These body parts seem to generate a void that the workers pick up and hence create more sexual larvae amongst existing brood pile. Queen's gasters were not sensed as a sign of queen's death probably because the suppression pheromones were stored here. Queenless replicates did not show significant differences in terms of mean numbers when compared with the queenright colonies. There-

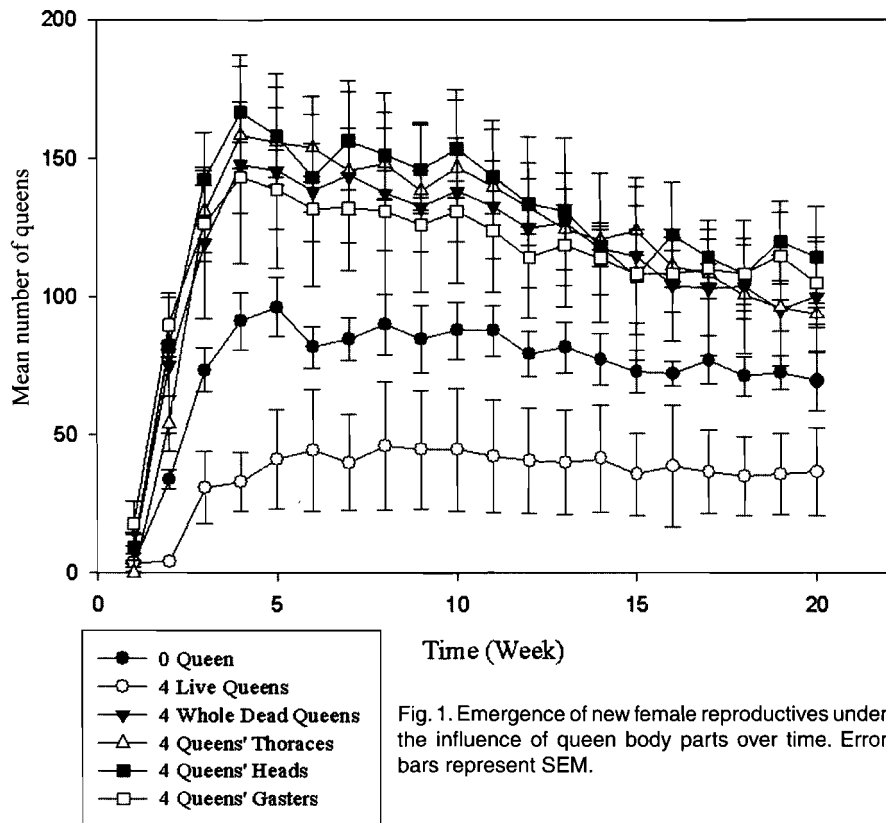


Fig. 1. Emergence of new female reproductives under the influence of queen body parts over time. Error bars represent SEM.

fore, death of queens could be a factor to trigger the offset of queen production in a colony.

However, emergence of male reproductives (Fig. 2) did not clearly portray a pattern for analysis. Most of them perished by Week 11 after the first batch of female reproductives emerged. In queenright colonies, the queens inhibit production of new reproductives by competing for "profertile" food that is needed most by developing sexual larvae (Buschinger & Kloft 1973) or by producing inhibitory pheromones (Berndt 1977). The presence of fertile queen alone is enough to generate instincts to kill sexual larvae in a colony (Edwards & Abraham 1991).

Queens exert their inhibitory effects differently in three phases of their lives. The young queens' ability is minimal as they have yet to mature in terms of egg-laying capacity. The mature queens have the most influential inhibitory effects. These mature queens also have visibly bigger gasters. When queens enter the final days of their lives,

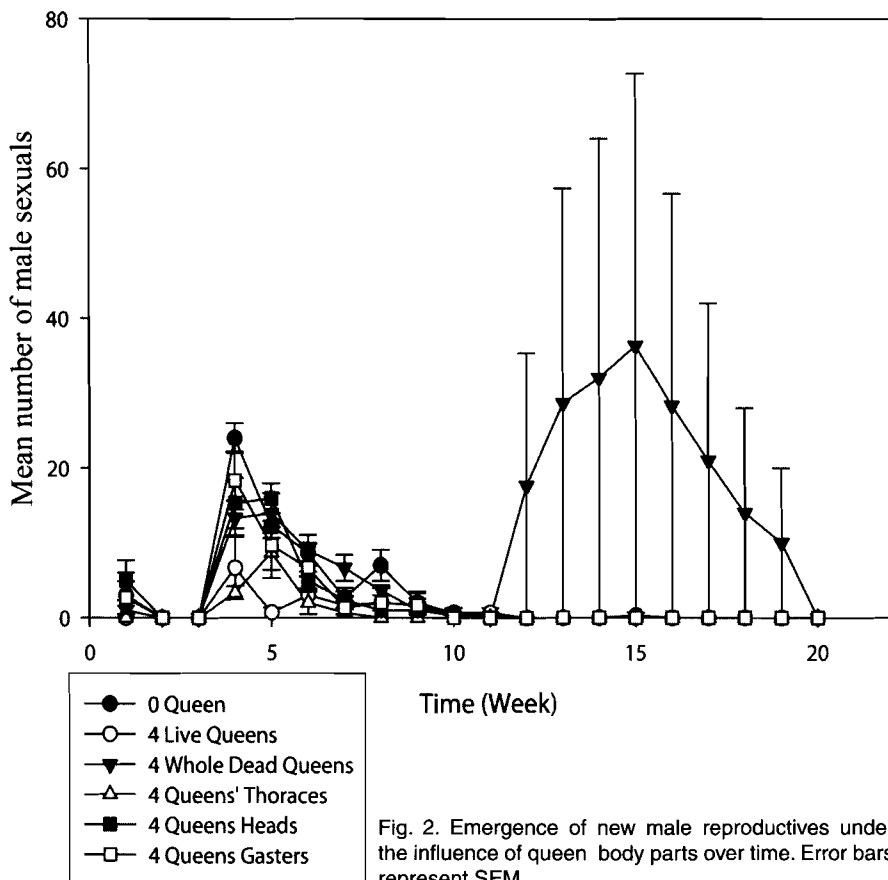


Fig. 2. Emergence of new male reproductives under the influence of queen body parts over time. Error bars represent SEM.

they become senile and possess zero capability in inhibition (Peterson-Braun 1975, Edwards 1984). It has also been demonstrated that virgin queens inhibit the production of reproductives to a certain extent (Berndt & Nitschmann 1979, Edwards 1984). In addition, Brian & Blum (1969) suggested that the queens seem to contain both the stimulatory and suppressive substances. Dead queens and extracts of queens of *Lasius alienus* and *Pheidole pallidula* were also shown to stimulate certain behavior patterns in workers, the most obvious being assembling, licking and transportation (Stumper 1956, reviewed in Brian & Hibble (1963). Brian & Blum (1969) further solidify queen's role in governing sex determination where workers of *Myrmica rubra* attacked female larvae but not if they are male, leading to the suggestion that the female larvae are sensed as potential rivals. In 1962, Carr showed

that dead queens replaced regularly were effective in suppressing larval growth and eliminating a behavioral mechanism for transmitting information to the workers and larvae. Carr later demonstrated that the mandibular gland of queens produced an attractive volatile chemical. These glands, when immersed in purified paraffin oil, increased larval growth rate (Brian & Hibble 1963). They also found whole queen heads crushed in ethanol and applied on larvae halved the amount of queen production whereas live queens reduced it to zero. All these observations have led to the proposition that queens contain both the stimulatory and suppressive substances.

Other body parts of the queen too have been shown to manifest effects on the production of reproductives. For instance, Vander Meer *et al.* (1982) suggested that the queen's postpharyngeal gland serves social functions in *S. invicta*. Vargo & Hulsey (2000) found that the head and the abdomen of *S. invicta* are equally attractive to the workers. Cogliatore & Cammaerts (1981) also found similar observations with *M. rubra* where the head, thorax and abdomen were all equally attractive in relation to their relative surface areas. Besides that, neocembrene produced in the Dufour's gland of the Pharaoh's ant queen may serve as a queen recognition compound (Edwards & Chambers 1984). Conversely, Cariou-Etienne *et al.* (1992) found that the thorax of the queens of *L. humile* was more attractive than the head or abdomen and they suggested that the metapleural gland or the epidermal cells in the thorax could be the source of the queen attractant.

Apart from new reproductives, queens are known to inhibit female reproductives from developing their ovaries and shedding their wings in the fire ant, *S. invicta* (Fletcher & Blum 1981, 1983). The queens produce a pheromone that inhibits workers from rearing male and female reproductives (Vargo & Fletcher 1986, 1987). In multiple-queen colonies, queen pheromones suppress egg production mutually among cohabiting queens (Vargo 1992).

Edwards & Abraham (1991) further proved the existence of queen's pheromonal control when introduced sexual larvae were cannibalized in the presence of queens. In *S. invicta*, queen pheromone induces workers to kill sexual larvae in the presence of a dealated queen. This pheromone is believed to have come from the poison sac, located in the gaster (Vargo & Hulsey 2000). Queen corpses were also found to have significant effects on stimulating execution of sexual larvae (Klobuchar & Deslippe 2002). This was in conformity with the results of this study where the dead queen's gasters were found to have inhibitory effects.

Rearing sexual larvae requires a mammoth amount of energy and resources. If a colony already consists of healthy and fertile sexuals, investment of such nature will drain the colony of resources. By having a system that regulates production of new sexuals, energy flow will be fully utilized and redistributed within the colony (Klobuchar & Deslippe 2002).

In the males, production was inconsistent and led to the conclusion that male sexuals are capable of inseminating the queens more than once in their relatively short lifespan. This finding was agreeable to Edwards' (1986) speculation where a single male was thought to mate up to four times. There is not much information available on this particular caste in ants as they have often been overlooked and understudied. Among the very little available preceding studies, Passera & Keller (1994) observed that the males of *L. humile* take flight into foreign colonies when opportunities are scarce in their mother colony. Their acceptance is largely based on the colony itself. When a colony is in need of increasing colony genetic diversity, the foreign males are readily accepted. Otherwise, they would be killed almost instantly. Death is usually inevitable in this species because queens generally bite the males violently to end mating (Keller & Passera 1992). However, the production of males or the effects of queens' presence were not discussed. There are still many fissures to be filled to comprehend the role of the males in ants. It is crucial that more studies are conducted to bridge the gap to further understand this phenomenon.

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REFERENCES

- Barker, J.F. 1978. Neuroendocrine regulation of oocyte maturation in the imported fire ant *Solenopsis invicta*. *General Comparative Endocrinology* 35: 234-237.
- Barker, J.F. 1979. Endocrine basis of wing casting and flight muscle histolysis in the fire ant *Solenopsis invicta*. *Experientia* 35: 552-554.
- Berndt, K.P. 1977. Physiology and reproduction in the pharaoh's ant (*Monomorium pharaonis* L.). I. pheromone mediated cyclic production of sexuals. *Wiadomosci Parazytologiczne* 23: 163-166.
- Berndt, K.P. & J. Nitschmann 1979. The physiology of reproduction in the Pharaoh's ant (*Monomorium pharaonis* L.). 2. The unmated queens. *Insectes Sociaux* 26: 137-145

- Bourke, A.F.G. 1988. Dominance orders, worker reproduction, and queen-worker conflict in the slave-making ant *Harpagoxenus sublaevis*. *Behavioral Ecology and Sociobiology* 23: 323-333.
- Brent, C.S. & E.L. Vargo 2003. Changes in juvenile hormone biosynthetic rate and the whole body content in maturing virgin queens of *Solenopsis invicta*. *Journal of Insect Physiology* 49: 967-974.
- Brian, M.V. 1979. Caste differentiation and division of labour. In: Hermann, H.R. (ed). *Social insects*, New York, Academic Press. Pp. 121-222.
- Brian, M.V. & J. Hibble 1963. 9-oxododec-trans-2-enoic acid and *Myrmica* queen extracts tested for influence on brood in *Myrmica*. *Journal of Insect Physiology* 9: 25-34.
- Brian, M.V. & M.S. Blum 1969. The influence of *Myrmica* queen head extracts on larval growth. *Journal of Insect Physiology* 15: 2213-2223.
- Buschinger, A. & W. Kloft 1973. Zur Funktion der Königin im sozialen Nahrungshaushalt der Pharaoameise *Monomorium pharaonis* (L.) (Hym., Formicidae) *Forschungsberichte des Landes Nordrhein-Westfalen*; Nr. 2306. Opladen: Westdeutscher Verlag, Pp. 34.
- Cariou-Etienne, A., S. Aron, & L. Passera 1992. Queen attractivity in the Argentine ant *Iridomyrmex humilis* (Mayr). *Behavioural Processes* 27: 179-186.
- Carr, C.A.H. 1962. Further studies on the influence of the queen in ants of the genus *Myrmica*. *Insectes Sociaux* 9: 197-211.
- Cogliatore, C. & M.C. Cammaerts 1981. Étude du pouvoir agrégatif des reines de *Myrmica rubra* L. *Insectes Sociaux* 28: 353-370.
- Edwards, J.P. 1984. Caste regulation and determination in the pharaoh's ant *Monomorium pharaonis* (L.). Ph.D. thesis, University of Southampton.
- Edwards, J.P. 1986. The biology, economic importance, and control of the pharaoh's ant, *Monomorium pharaonis* (L.). In: Vinson, S.B. (ed) *Economic impact and control of social insects*, New York, Praeger Publishers, Pp. 257-271.
- Edwards, J.P. 1987. Caste regulation in the pharaoh's ant *Monomorium pharaonis*: the influence of queens on the production of new sexual forms. *Physiological Entomology* 12: 31-39.
- Edwards, J.P. & J. Chambers 1984. Identification and source of a queen-specific chemical in the Pharaoh's ant, *Monomorium pharaonis* (L.). *Journal of Chemical Ecology* 10: 1731-1747.
- Edwards, J.P. & L. Abraham 1991. Caste regulation in the pharaoh's ant *Monomorium pharaonis*: recognition and cannibalism of sexual brood by workers. *Physiological Entomology* 16: 263-271.
- Fletcher, D.J.C. & M.S. Blum 1981. Pheromonal control of dealation and oogenesis in virgin queen fire ants. *Science* 212: 73-75.
- Fletcher, D.J.C. & M.S. Blum 1983. Regulation of queen number by workers in colonies of social insects. *Science* 219: 312-314.
- Fletcher, D.J.C. & K.G. Ross 1985. Regulation of reproduction in eusocial Hymenoptera. *Annual Review of Entomology* 30: 319-343.

- Higo, H.A., S.J. Colley, M.L. Winston, & K.N. Slessor 1992. Effects of honey bee queen mandibular gland pheromone on foraging and brood rearing. *Canadian Entomologist* 124: 409-418.
- Hölldobler, B. & E.O. Wilson 1990. *The Ants*. Cambridge, Massachusetts, Harvard University Press, Pp 732.
- Katzav-Gozansky, T., V. Soroker, & A. Hefetz 2002. Evolution of worker sterility in honey bees: egg laying workers express queen-like secretions in Dufour's gland. *Behavioral Ecology and Sociobiology* 51: 588-589.
- Keller, L. & L. Passera 1992. Mating system, optimal number of matings, and sperm transfer in the Argentine ant *Iridomyrmex humilis*. *Behavioral Ecology and Sociobiology* 31: 359-366.
- Klobuchar, E.A. & R. J. Deslippe 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). *Naturwissenschaften* 89: 302-304.
- Mehdiabadi, N.J., H.K. Reeve, & U.G. Mueller 2003. Queens versus workers: sex-ratio conflict in eusocial Hymenoptera. *Trends in Ecology & Evolution* 18: 88-93.
- Pankiw, T., Z.Y. Huang, M.L. Winston, & G.E. Robinson 1998. Queen mandibular gland pheromone influences worker honey bee (*Apis mellifera* L.) foraging ontogeny and juvenile hormone titers. *Journal of Insect Physiology* 44: 685-692.
- Passera, L. 1969. Contribution a l'etude du determinisme de la caste chez une fourmi: *Plagiolepis pygmaea* (Latr.). In: *Proceedings of the International Union for the Study of Social Insects*. Pp. 233-238.
- Passera, L. 1980. La fonction inhibitrice des reines de la fourmi *Plagiolepis pygmaea* (Latr.): rôle des pheromones. *Insectes Sociaux* 27: 212-225.
- Passera, L. & L. Keller 1994. Mate availability and male dispersal in the Argentine ant *Lineithema humile* (Mayr) (= *Iridomyrmex humilis*). *Animal Behaviour* 48: 361-369.
- Passera, L., S. Aron, E.L. Vargo, & L. Keller 2001. Queen control of sex ratio in fire ants. *Science* 293: 1308-1310.
- Peacock, A.D., I.C. Smith, D.W. Hall, & A.T. Baxter 1954. Studies in Pharaoh's ant, *Monomorium pharaonis* (L.), 8: Male production by parthenogenesis. *Entomologists' Monthly Magazine* 90: 154-158.
- Peacock, A.D., J.H. Sudd, & A.T. Baxter 1955. Studies in Pharaoh's ant, *Monomorium pharaonis* (L.). 11. Colony foundation. *Entomologists' Monthly Magazine* 91: 125-129.
- Peterson-Braun, M. 1975. Untersuchungen zur sozialen Organisation der Pharaoameise *Monomorium pharaonis* (L.) (Hymenoptera, Formicidae). I. Der Brutzyklus und seine Steuerung durch populationseigene Faktoren. *Insectes Sociaux* 22: 269-291.
- Sakagami, S.F. 1958. The false queen: the fourth adjustive response in dequeened honey bee colonies. *Behaviour* 13: 280-296.
- Slessor, K.N., L. Kaminski, G.G.S. King, J.H. Bordon, & M.L. Winston 1988. Semiochemical basis of the retinue response to honey bee queens. *Nature* 332: 354-356.

- Sole, C.L., P. Kryger, A. Hefetz, T. Katzav-Gozansky, & R.M. Crewe 2002. Mimicry of queen Dufour's gland secretions by workers of *Apis mellifera scutellata* and *A. m. capensis*. *Naturwissenschaften* 89: 561-564.
- Stumper, R. 1956. Sur les secretions attractives des fourmis femelles. *Comptes rendus de l'Académie des sciences* 242: 2487-2489.
- Vander Meer, R.K., B.M. Glancey, C.S. Lofgren, A. Glover, J.H. Tumlinson, & J. Rocca 1980. The poison sac of red imported fire ant queens: Source of a pheromone attractant. *Annals of the Entomological Society of America* 73: 609-612.
- Vander Meer, R.K., B.M. Glancey & C.S. Lofgren 1982. Biochemical changes in the crop, oesophagus and postpharyngeal gland of colony-founding red imported fire ant queens (*Solenopsis invicta*). *Insect Biochemistry* 12: 123-127.
- Vargo, E.L. 1992. Mutual pheromonal inhibition among queens in polygynous colonies of the fire ant *Solenopsis invicta*. *Behavioral Ecology and Sociobiology* 31: 205-210.
- Vargo, E.L. 1997. Poison gland of queen fire ants (*Solenopsis invicta*) is the source of a primer pheromone. *Naturwissenschaften* 84: 507-510.
- Vargo, E.L. 1998. Primer pheromones in ants. R.K. Vander Meer, M.D. Breed, K.E. Espelie, and M.L. Winston, (eds.), *In: Pheromone communication in social insects ants, wasps, bees, and termites*. Boulder, Colorado, Westview Press. Pp. 293-313.
- Vargo, E.L. 1999. Reproductive development and ontogeny of queen pheromone production in the fire ant *Solenopsis invicta*. *Physiological Entomology* 24: 370-376.
- Vargo, E.L. & D.J.C. Fletcher 1986. Evidence of pheromonal queen control over the production of male and female sexuals in the fire ant, *Solenopsis invicta*. *Journal of Comparative Physiology A* 159: 741-749.
- Vargo, E.L. & D.J.C. Fletcher 1987. Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. *Physiological Entomology* 12: 109-116.
- Vargo, E.L. & L. Passera 1991. Pheromonal and behavioral queen control over the production of gynes in the Argentine ant *Iridomyrmex humilis* (Mayr.). *Behavioral Ecology and Sociobiology* 28: 161-169.
- Vargo, E.L. & M. Laurel 1994. Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. *Journal of Insect Physiology* 40: 601-610.
- Vargo, E.L. & C.D. Hulsey 2000. Multiple glandular origins of queen pheromones in the fire ant *Solenopsis invicta*. *Journal of Insect Physiology* 46: 1151-1159.
- Wilson, E.O. 1971. *The Insect Societies*. Cambridge, Massachusetts, Harvard University Press. Pp. 548.
- Winston, M.L. & K.N. Slessor 1992. The essence of royalty: honey bee queen pheromones. *American Scientist* 80: 374-385.

- Winston, M.L., K.N. Slessor, L.G. Willis, K. Naumann, H.A. Higo, M.H. Wyborn, & L.A. Kaminski 1989. The influence of queen mandibular gland pheromones on worker attraction to swarm clusters and inhibition of queen rearing in the honey bees (*Apis mellifera* L.). *Insectes Sociaux* 36: 15-27.
- Winston, M.L., H.A. Higo, & K.N. Slessor 1990. Effect of various dosages of queen mandibular gland pheromone on the inhibition of queen rearing in the honey bee (Hymenoptera: Apidae). *Annals of the Entomological Society of America* 83: 234-238.
- Winston, M.L., H.A. Higo, S.J. Colley, T. Pankiw, & K.N. Slessor 1991. The role of queen mandibular pheromone and colony congestion in honey bee (*Apis mellifera* L.) reproductive swarming. *Journal of Insect Behavior* 4: 649-659.

