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GONADAL DEVELOPMENT IN WINGED FORMS OF ANTS (HYMENOPTERA: FORMICIDAE) WITH APPLICATION TO MANAGEMENT

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Abstract Development of the reproductive stages in the life cycle of ants varies among species. Formicine ants usually overwinter or spend development time in the nest between eclosion and the mating flight. Many of the Dolichodorinae and Myrmecinae mate in the nest and colonies reproduce by budding. A formicine example, *Camponotus modoc*, emerges as winged forms in August and remains in the colony until swarming the following spring. The emergence of reproductives in structural infestations throughout the winter months creates management concerns as winged forms, particularly males, make sporadic weak fights to windows or lighted areas. Reproductive systems of males and winged females were examined following eclosion and at three-month intervals until the spring nuptial flight to determine development of sexual organs. Spermatogenesis was complete by emergence, with no further development of the male gonads and apparent shrinkage of the testes in the first week following eclosion. Ovariole number varies among species of *Camponotus*. Each ovary of *C. modoc*, composed of 48-50 ovarioles, contained oocytes with varying degrees of development through the winter until by spring the terminal ovum in many ovarioles had completed vitellogenesis. Oogenesis and vietllogenesis occur throughout the life of the queen. Winged females and males occur in structures particularly in winter months before the nuptial flight and challenge management strategies in inspections for location and treatment.
Key words Carpenter ants, ovaries, testes, management

INTRODUCTION

Identification

Urban pest ants fall into four subfamilies: Formicinae, Dolichodorinae, Myrmicinae, and Ponerinae. These ants are found worldwide and have been introduced throughout the world. Oi and Vail (2011) cited 30 pest ants in 21 genera with a generous approximation of 13% of the North American ant species. Klotz et al. (2008) named 25 genera with seven in subfamily Formicinae, six in Dolichodorinae, ten in Myrmicinae, and two in Ponerinae. In comparison of exotic ants introduced into North America, 27 species in 14 genera have been identified. Ants introduced into Europe include 16 species in 11 genera (Klotz et al., 2008). These introduced, exotic, or tramp species can become serious pests. Nine genera have been recorded as introduced into both North America and Europe. Ant identification has taken on a worldwide perspective. Management of pest ants relies first on the proper identification and second on the knowledge of the biology of specific ants. Variations in life histories within a species differ with latitude and with specific locations. Keys in books, publications, the Internet, and research institutions are available for correct identification to species. Following proper identification, biological features of the species provide clues to management. Knowledge of reproductive activity is critical to understanding the biology.

Reproduction

Polygynous colonies reproduce by fission or budding when one or more of the many queens leave the nest along with workers and brood to establish a new colony. Pest species belonging to the

Dolichodorinae and Myrmicinae commonly establish new colonies by budding (Klotz et al., 2008). Because these colonies share the same gene pool, these open societies lack intraspecific aggression. Many exotic or tramp ants fall into this category and include: *Linepithema humile* (Mayr), *Tapinoma melanocephalum* (Fabricius), *T. sessile* (Say)*, and Monomorium pharaonis* (L.). In some colonies flights occur at irregular times or mating occurs within the nests. Queens in polygynous colonies are short-lived, usually less than one year (Passera, 1994).

Colonies of formicine species generally reproduce through mating flights where winged males and females take flight in a synchronized fashion following specific environmental conditions. Winged reproductives are an energy expense to the colony and are more common in northern latitudes. In three pest genera of formicine ants (*Camponotus, Prenolepis* and some species of *Lasius*), reproductives overwinter in the nest and the nuptial flights occur the following spring (Klotz et al., 2008). Many formicine queens in monogynous colonies live for many years.

Life History

Carpenter ants remain the most common structurally damaging insect in northern United States and Europe (Akre et al., 1995). Although some species are nuisance pests in urban environments, a number of species are classified as structurally damaging: *Camponotus modoc* Wheeler and *C. vicinus* Mayr in western half of North America, *C. pennsylvanicus* (DeGeer) and *C. chromaiodes* Bolton in the eastern half of North America, *C. herculeanus* (L.) in both northern Europe and northern North America, and *C. ligniperda* Latreille in northern Europe are most commonly identified (Butovitsch, 1976). Altogether 24 species of *Camponotus* spp. have been recorded as either nuisance or structurally damaging in North American and Canada (Hansen and Klotz, 2005). Depending on latitude, carpenter ant colonies enter diapause in September or October and break diapause from March through June (Klotz et al., 1999). Although differences occur in life history of each species, homeowners are most aware of infestations in structures when the mating flights occur between April and June. If nests occur within structures winged males and females are attracted to windows and lights as they begin their nuptial flight. Males emerge before females often as early as December when temperatures in structures are higher than temperatures in the natural environment. These ants generally return to nests and remain in overwintering sites until conditions warrant the spring flight.

Carpenter ant colonies are monogynous and nests producing winged forms may be found in either parent or satellite nests. A parent or main nest contains the queen, workers, brood, and winged forms. A satellite contains all but the queen. Usually parent nests are located in a natural area, outside a structure, while satellite nests may occur within structures (Hansen and Akre, 1985). Winged forms are produced when colonies are mature, usually over eight to ten years old, in the late summer and overwinter in either the parent nest or satellite nest until the following spring. These overwintering winged adults are perceived by homeowners to be the damaging stages and management of these forms is imperative (Cannon and Fell, 1990) (Fowler, 1985).

Female Reproductive System

The female reproductive system consists of a pair of ovaries, each composed of ovarioles; the oviduct; spermatheca; and accessory glands. Each ovariole consists of a terminal filament that supports the ovary, the egg tube where oogenesis occurs and the pedicel that unites the ovariole with the lateral oviduct. The spermatheca located on the common oviduct serves as the sperm storage organ once the female has been inseminated. The polytrophic ovarioles have nurse cells or trophocytes associated with each developing egg. Oogenesis starts when the germ cells at the terminal end of the ovariole produce the oocyte and trophocytes. Oocytes grow in the vitellarium as nurse cells deposit yolk and the mature eggs are forced down the egg tube by the continuous development of more oocytes and nurse cells. The oocyte completely absorbs the nutriments from the nurse cells to produce the mature egg at the end of the ovariole. An ovariole resembles a string of beads increasing in size with the alternation of egg chambers and nutritive cells as they progress through the egg tube (Wigglesworth, 1972) (Snodgrass, 1956).

Male Reproductive System

The male reproductive system consists of a pair of testes composed of follicles, the vas deferens, seminal vesicles, and accessory glands. Spermatogenesis occurs in the follicles during the larval and pupal stages (Snodgrass, 1956) and the male ant ecloses with all the sperm that he is ever going to produce (Hölldobler and Bartz, 1985). Spermatogonia develop into spermatocysts and are transformed into mature spermatozoa where they are stored in the seminal vesicles. Degeneration of the testes and movement of spermatozoa into seminal vesicles follows eclosion (Wheeler and Krutzsch, 1992). Two large curved accessory glands are attached to the posterior of the seminal vesicles (Snodgrass, 1956) and produce secretions that mix with spermatozoa to form spermatophores (Wigglesworth, 1972).

The objective of this paper is to review reproductive strategies by examining development of reproductive structures and to relate these and life histories to management of urban pest ants, particularly carpenter ants. Over-wintering colonies in the laboratory, inspecting infestations in structures, and examining the development of the winged males and females before the nuptial flights have provided additional information on the development of winged reproductives.

MATERIALS AND METHODS

Overwintering Females and Males

Development of ovaries and testes in the winged females and males of *C. modoc* were studied at three month intervals: fall, winter, and spring (Witherell, 1991). *C. modoc* nests containing workers and brood were collected in May and maintained in the laboratory. Males and winged females emerged from pupae in the nest during the first week in August. In October, January and May males and winged females were randomly selected from these nests for dissection and examined for changes in development (Table 1). Dissections were made with 0.75% NaCl and reproductive organs from both males and winged females were removed and placed in alcoholic Bouin's fixative, dehydrated with ethanol and butanol and embedded in paraffin as described by Gatenby and Beams (1950). Tissues were sections at 12 μ, transferred to slides, and stained with Delafield's hematoxylin and Eosin B.

Table 1. Winged *Camponotus modoc* dissected for examination of reproductive structures.

Sex	No.	Collection site	Gross Dissect	Histology	Season
female	12	Spokane Co	21-Oct	21-Oct	fall
	12	Spokane Co	20-Jan	20-Jan	winter
	3	Spokane Co	26-May	26-May	spring
	2	Clallam Co	26-May	26-May	spring
males	12	Spokane Co	21-Oct	21-Oct	fall
	3	Whitman Co	10-Oct	10-Oct	fall
	5	Clallam Co	20-Jan	20-Jan	winter
	4	Clallam Co	1-Mar	1-Mar	spring

Dissections of Males and Winged Females for Morphology
Males and winged females were selected from nests collected in June and maintained in the laboratory. Ants were dissected from nests in early August before and following eclosion. Winged females of other *Camponotus* spp. also were collected and dissected, with emphasis on recording ovariole numbers. Dissections were made in 10% ethanol with methylene blue. Fat bodies and tracheae were removed exposing respiratory, digestive and reproductive systems for gross examination.
Infestations in structures involving winged forms of *Camponotus* spp.
Notes on 210 inspections of infestations for five years (2006-2011) were reviewed to determine the occurrence and timing of winged males and females observed by homeowners within or on the exterior of the structure. Infestations were selected from referrals made by extension personnel and through personal contacts. On inspections, attempts were made to locate parent and satellite nests or to determine conducive conditions where nests might be located.

RESULTS AND DISCUSSION

Overwintering Females and Males
Gross dissection of males. The adult male reproductive system of *C. modoc* includes a pair of testes that are small globular structures (0.3 mm) with tracheae intertwined. The tracheae appear to add structural support anchoring testes to the body wall. Seminal vesicles are tubes leading from the testes to accessory glands and have a diameter 0.1 mm and 3.5 mm long. The distal third of the seminal vesicles are coiled. The proximal ends are constricted as each enters the ventral surface of the accessory gland. These structures are large sacs (l.3 mm x 0.5 mm) that are milky white. The posterior ends of the accessory glands fuse to form the ejaculatory duct. In comparison of the male reproductive tissue from October to January to May, differences were not observed. In fall dissections the fat body was opaque, pearly white, in spring dissections the fat body was reduced in size and translucent (Witherell, 1991).
Gross dissection of winged females. Organs dissected from *C. modoc* gynes included the ovaries that consist of numerous white thread-like structures, the ovarioles (4 mm in length). The posterior end of each ovariole is wider than the anterior and some ovarioles are bead-like in appearance. Tracheae were highly intertwined with the ovarioles and appear to help support the ovary structure by anchoring the ovary to the body wall. The ovarioles terminate in the lateral oviduct. The pair of lateral oviducts unites at their posterior ends forming the common medial oviduct. In the winter and spring dissections, the size and number of 'beads' in the ovarioles increased. The number of ovarioles containing eggs (beads) also increased in winter and spring dissections. The fat body occupied a major portion of the gaster cavity and surrounded the digestive and reproductive systems. The fat body became more translucent in the spring dissection. The changes in appearance are similar to changes reported by Cannon and Fell (1990). They investigated *C. pennsylvanicus* and found that lipids and proteins are consumed during the over-wintering phase.
Histological examination of males. Corresponding to the gross dissections of the male reproductive system in *C. modoc*, there were no histological changes during these time intervals. Remnants of the testes were all that remained in the adult. The seminal vesicles were filled with spermatozoa producing a spiral pattern. The large accessory glands were filled with secretions of proteins and carbohydrate to mix with spermatozoa in the formation of spermatophores (Wigglesworth, 1972) (Wheeler and Krutzsch, 1992). Folds at the posterior ends of the accessory glands were observed in the spring specimens of *C. modoc*. The number of follicles in each testis was nine for *C. modoc* (Figure 1) but varies with other *Camponotus* spp. *C. pennsylvanicus* has 12-16 follicles; *C. ligniperda* has 17 follicles

(Forbes 1954) *C. festinatus* (L.), and *C. sayi* Emery, both possess 9 follicles and *C. mina* Forel has 10-11 follicles (Wheeler and Krutzsch, 1992). *Lasius niger* (Buckley) possesses 7 follicles (Forbes, 1954). In *C. herculeanus* and *C. ligniperda,* spermatogenesis is not yet completed at the time of ecdysis but continues up to 25 days before migrating into the seminal vesicles (Hölldobler, 1966).

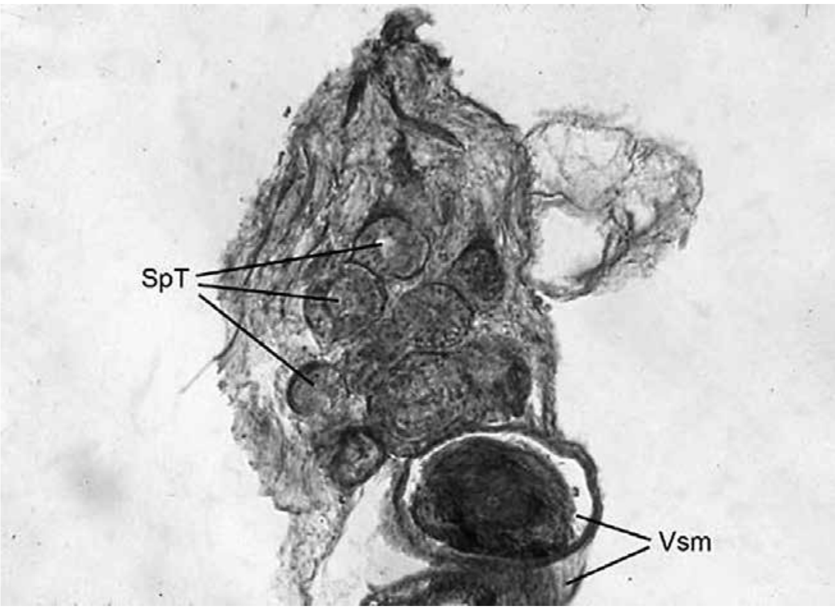

Figure 1. Histological section of testis of *Camponotus modoc* male showing nine spermatic tubes or follicles (SpT) and beginning of the seminal vesicle (Vsm) on a fall dissection.

Histological examination of winged females. Cross-sections of the *C. modoc* ovaries showed that each was composed of 48-50 ovarioles. Individual follicles at the proximal end of a number of ovarioles were observed in sections from each season. In ovarioles of fall gynes, developing oocytes (75 μ) with their accompanying trophocytes were vestigial (Figure 2). In ovarioles of the winter gynes, the ovum had increased in size, due to partial absorption of nurse cells (Figure 3). The terminal ovum (310 μ) in spring dissections was larger in size due to vitellogenesis. Trophocytes of spring eggs had been absorbed leaving only the mature ovum in the follicle at the proximal end of the ovariole (Figure 4).

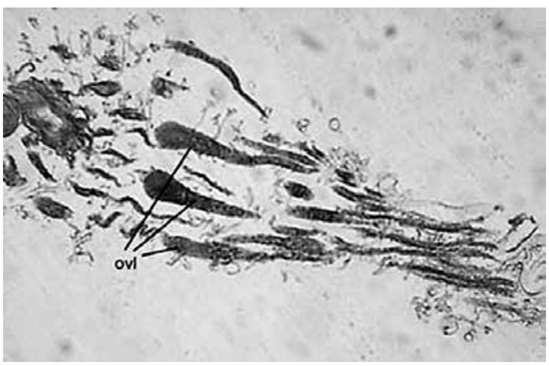

Figure 2. Longitudinal section of *Camponotus modoc* winged female showing ovarioles (Ovl) in a fall dissection.

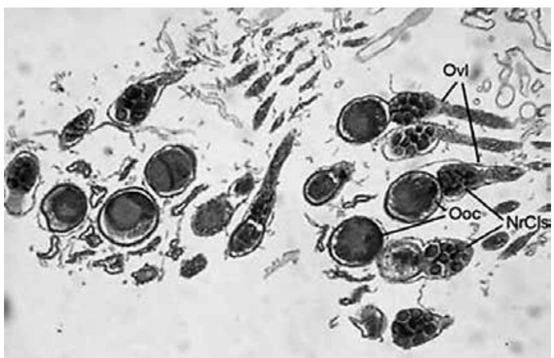

Figure 3. Longitudinal section of *Camponotus modoc* winged female showing ovarioles (Ovl), oocytes (Ooc), and nurse cells (NrCls) in a winter dissection.

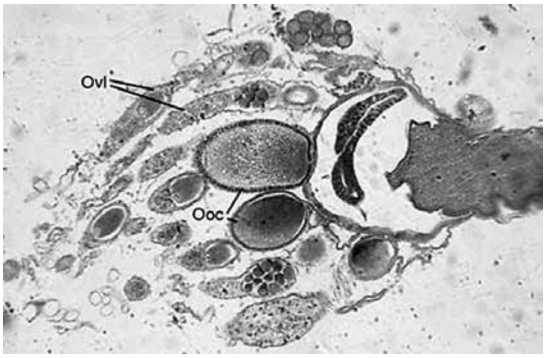

Figure 4. Longitudinal section of *Camponotus modoc* winged female showing terminal oocyte (Ooc) in an ovariole (0vl) in a spring dissection.

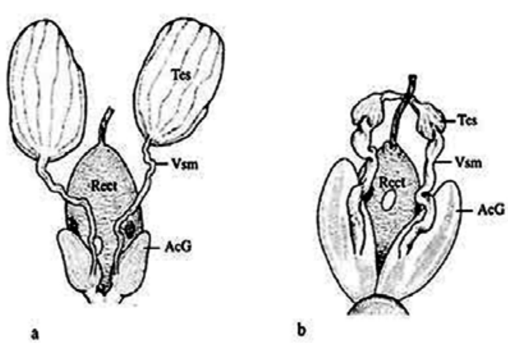

Figure 5. Reproductive systems in male *Camponotus modoc* at (a) 1 day and (b) 1 week following eclosion showing accessory gland (AcG), seminal vesicle (Vsm), testis (Tes). These lie near the rectum (Rect).

Dissections to Study Morphology

Winged females. Numbers of ovarioles per ovary varied among the *Camponotus* species examined: 13 to 48 with the highest numbers occurring in *C. modoc* (Table 2). Number of ovarioles may be linked to size of colonies and polygyny as the largest colonies are *C. modoc* and the only polygynous species is *C. vicinus* (Akre et al., 1994).

Table 2. Ovariole number in winged females of Camponotus spp.

Camponotus spp.	No. dissected	Range	Ave. No.
C. essigi M.R. Smith	4	12-16	13
C. herculeanus (L.)	2	43-45	44
C. modoc Wheeler	14	46-52	48.3
C. noveboracensis (Fitch)	1	44	44
C. pennsylvanicus (DeGeer)	4	30-38	34
C. semitestaceus Snelling	1	22	22
C. vicinus Mayr	2	20-22	20.5

Males. Dissections of males made on day one and day seven following eclosion revealed the testes had become drastically reduced after one week. On day one the testes were enlarged and connected to narrow seminal vesicles and small accessary glands. By day seven the testes were reduced and the seminal vesicles and accessory glands were enlarged (Figure 5).

Structural Infestations

Between 2006 and 2011, 210 structures were inspected for carpenter ant infestations in the Pacific Northwest (Table 3). Workers were observed in all infestations and winged forms were observed in

45%. In the months of January-February and November-December very few workers were observed. Workers were found where water was available, in bathrooms and kitchens. In the months of March through October, the numbers of workers were higher when foraging occurred outside the structures. Highest numbers of winged ants were observed from March through June; 33% of the structures had winged ants during the period from eclosion to swarming (September-February).

Table 3. Number infestation sites in 2006-2011 with *Camponotus modoc*, *C. vicinus*, or *C. essigi* where males, winged females or both winged forms were observed.

Months	*C. modoc*			*C. vicinus*			*C. essigi*		
	males	females	Both	males	females	Both	males	females	Both
Jan-Feb	4	0	1	3	0	0	5	3	3
Mar-Apr	8	4	1	5	0	0	6	3	3
May-Jun	6	8	3	5	3	2	0	0	0
July-Aug	0	0	0	0	3	3	0	0	0
Sep-Oct	0	0	0	0	0	0	1	2	2
Nov-Dec	3	0	0	1	0	0	2	1	0
Total	21	12	5	14	6	5	14	9	8

Winged forms of carpenter ants often over-winter in satellite nests that commonly occur in structures. Whereas in a natural environment these forms generally do not appear until the nuptial flight, in heated structures, males and often females leave over-wintering sites and appear at windows in an attempt to begin a flight. Males stimulate flight by the pheromone produced in the mandibular gland (Hölldobler and Maschwitz, 1965). This pheromone appears to be released only when the environmental conditions for flight are established. When the pheromone is not released winged forms usually return to over-wintering sites.

CONCLUSIONS

Dissections and histological sections of adult males of *C. modoc* in fall, winter and spring showed no development of gonadal tissues between the time of eclosion and swarming. However, spermatozoa were moved from the testes to the seminal vesicle during the first week following eclosion. C. *modoc* winged females showed considerable development throughout the period from eclosion in summer and mating flights the following spring with the development of mature oocytes. Ovariole numbers varied among *Camponotus* species studied with 48-50 found in *C. modoc.*

In management of structural infestation, eliminating these winged forms can be difficult (Hansen, 2007). Baits are not effective, as the winged forms are not foraging. The ants may be isolated in small satellite nests under insulation, in attics, or other voids. Finding these nests to directly apply dusts, aerosols, or sprays can be challenging. Winged forms produce 'rustling' noises when disturbed and drumming on the void or ceiling may elicit this response and the treatment can be confined to the affected void. Drilling into voids may be necessary to locate satellite nests with reproductives.

Understanding this phase in the biology of carpenter ants is helpful to understanding the infestation and explaining the problem to clients. The winged forms are developing and waiting for the proper environmental conditions for nuptial flights before leaving the site to mate and establish new colonies. The winged forms do not cause damage, but workers that are present with the winged forms in satellite nests may cause damage. These ants are attracted to water sources in structures and liquid or gel baits may be effective. Follow-up inspections during the foraging season are prescribed because parent nests are located outside the structure and additional satellite nests may be located during the next overwintering season.

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