

# Immature Development and Colony Growth of Crazy ant *Paratrechina fulva* Under Laboratory Conditions (Hymenoptera: Formicidae)

by

Angela María Arcila<sup>1</sup>, Luis Antonio Gómez<sup>2</sup>, & Patricia Ulloa-Chacón<sup>3</sup>

## ABSTRACT

An account is given on some aspects of the biology of crazy ant *Paratrechina fulva* (Mayr). The number of larval instars for the male and worker castes were determined using a sample of larvae collected from the field. Head width and body length were measured; workers went through three larval instars while males did it through four. Three kinds of experimental colonies hexagynous, trigynous and monogynous were set up. The immature development of workers and the growth of a colony under laboratory conditions were studied. The mean duration of egg, larva and pupa was 16.2, 10.8 and 12.2 days respectively. The total immature development took from 23 to 50 days with a mean of 39.2 days. The highest production of brood was obtained in the hexagynous colonies. The highest production of eggs was reached faster in the polygynous colonies in contrast with the monogynous, while the maximum number of larvae and pupae was obtained towards the end of the observation period for all of the colonies. Brood mortality was higher during the incubation period and it reached an average of 70% for the monogynous colonies, this percentage was lower in the development of larvae to pupae reaching nearly 50% for the three kinds of colonies. Significant differences were found between the mortality percentages of larvae during incubation for the three kinds of colonies studied, specifically between the monogynous and hexagynous colonies. The polygynous colonies therefore proved to be more stable experimental units, showing a lower percentage of mortality of larvae after incubation and, although no significant difference was found, polygynous colonies were more successful in rearing brood to the pupal stage and they reached and maintained a pupae/eggs rate of at least 50% faster than the monogynous colonies.

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<sup>1</sup>Departamento de Biología, Facultad de Ciencias, Universidad del Valle, P.O. Box: 25360, Cali, Valle, Colombia, e-mail: angelarcila@hotmail.com

<sup>2</sup>Centro de Investigación de la Caña de Azúcar de Colombia, CENICANA, P.O. Box 9138, Cali, Valle, Colombia, e-mail: lagomez@cenicana.org

<sup>3</sup>Departamento de Biología, Facultad de Ciencias, Universidad del Valle, P.O. Box: 25360, Cali, Valle, Colombia, e-mail: pachacon@uniweb.net.co Coresponding author.

**Key words:** Crazy ant, *Paratrechina fulva*, larval instars, immature development, colony growth.

## INTRODUCTION

The crazy ant (CA) *Paratrechina fulva* was introduced to Colombia almost 30 years ago. Apparently it was brought from Brazil as a control agent for leaf-cutting ants (*Atta* sp.) and venomous snakes (Zenner-Polanía 1990a), and was first detected as a pest in 1971 at Puerto Boyacá (Central Colombia) (ICA 1972). At present, it is distributed over a vast extension of the country (Santander, Boyacá, Cundinamarca and Valle del Cauca) and covers a wide altitudinal gradient from 150m to 2600m above sea level.

This ant has become an ecological and agricultural problem. When an area is invaded by *P. fulva* it displaces native fauna, particularly other ants, therefore decreasing its diversity (Zenner-Polanía & Martínez, 1992, Aldana *et al.* 1995). In agroecosystems the main trouble comes from its association with homopteran insects that are directly responsible for crop damage.

Little is known about the biology of *P. fulva*. Most of the studies that have been carried out deal with the problem of its control and sampling techniques (Zenner-Polanía & Ruiz 1982, Zenner-Polanía 1990b, Chacón de Ulloa *et al.* 1994, Gómez & López-Pulido 1995, Gómez & Lastra 1997, Gutiérrez & Calderón 1997, Chacón de Ulloa *et al.* 2000). Furthermore the few studies that exist about the basic biology of this ant are based on topics such as habitat, feeding preferences and symbiotic relationships with other arthropods (Zenner-Polanía & Ruiz 1985, Zenner-Polanía 1990a).

Considering the importance of biological data as a way to improve control strategies and/or implement new ones, the present investigation is an attempt to fulfill the existing information gap on the biology of CA. We contribute to the knowledge of the larval instars of the worker caste and males, the immature development of the former and some aspects of colony growth under laboratory conditions that will help us to establish experimental units for future control studies.

## MATERIALS AND METHODS

### **Collecting ants from field colonies**

*P. fulva* ants do not build elaborate nests, instead they take advantage of preformed cavities or almost any empty space as long as there is adequate moisture and warm temperature (Zenner-Polanía 1990a). When dry conditions prevail, they are usually found below the soil surface, whereas under wet weather it is more common to find them on

the soil surface below decaying vegetable material (Aldana *et al.* 1995).

Collections were made during June 1998 at Hacienda San Antonio (Buga -Valle del Cauca). Nests were found under dead leaves gathered between the roots of the trees. The material obtained was taken to the laboratory and separated from the leaves by flooding.

### **Establishing laboratory colonies**

Laboratory rearing of *P. fulva* was initiated with adult queens and workers collected in the field. Ants were kept in plastic boxes (33x26x10cm) at 27°C and 80% R.H. Their interior vertical surfaces were coated with Fluon® (Polytetrafluoro-ethylene, ICI Fluoropolymers INC, Exton, P.A. 19341) to keep ants from escaping. Inside the boxes artificial nests made of plaster as described by Passera *et al.* (1988) were set up. Colonies were fed with pure water, 50% honey-water solution, tuna fish and chopped *Diatraea saccharalis* larvae and entire adults or cockroaches.

Three kinds of experimental colonies were set up. They were composed by one, three or six queens and a fixed volume of 3ml (approximately 900 individuals) of workers per queen. There were three replicates of each treatment for a total of nine colonies.

### **Larval instars of the worker caste and males**

To determine the number of larval instars of the worker caste and males, a sample containing 178 larvae was examined. Nests were found at Hacienda San Antonio and the collected brood was stored in 80% ethanol. Total body length and head capsule widths were measured using a binocular stereoscope with a micrometer. Other observations concerning the external appearance of the larvae and their mandibles were made as well.

### **Immature development of the worker caste**

In order to study the immature development of the worker caste, observations were made on the nine experimental colonies previously described. To avoid mixing different generations in the same colony, the maturation of the first eggs oviposited by the queen(s) was allowed until they reached the larval stage. Once this was accomplished new eggs were taken out. In this way, it was possible to follow the development of just one cohort at a time. Individuals were counted every third day in each colony, obtaining information about the time span between egg, larva and pupa stages.

### **Colony growth under laboratory conditions**

To study the development of a colony through time, colonies previously described were employed, they will be referred as hexagynous (six

queens), trigynous (three queens) and monogynous (one queen) colonies.

Once observations on the immature development of the worker caste were finished, the remaining brood was discarded leaving only the queen(s) and adult workers. These colonies were then observed during 10-11 weeks and the number of egg masses, larvae and pupae were recorded every third day.

## RESULTS AND DISCUSSION

### Larval instars of the worker caste and males

*Measurements of larvae.* Dyar's (1890) principle was used to determine the number of instars by measuring head capsule width of the collected larvae. This variable showed only four values (0.20, 0.27, 0.33

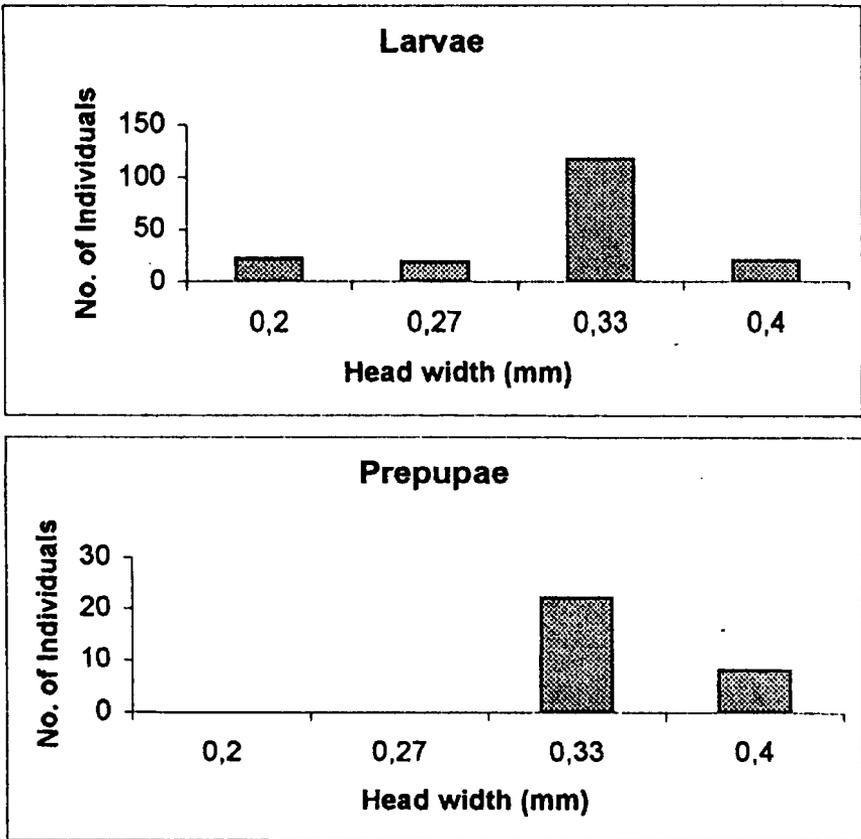


Fig. 1. Distribution of head widths and prepupae found in a colony with the male brood collected at Hacienda San Antonio (Buga, Valle).

and 0.40mm) suggesting the existence of four larval instars, but we still had to separate the workers and males.

Male brood was commonly observed in the field throughout the year and their larvae were very difficult to distinguish from the worker ones because they are about the same size and shape. We measured the head width of some prepupae and found two values that coincided with those of the larval instars III and IV (Fig. 1). We observed then their mandibles under a binocular microscope and found two different types. Apparently the larval instar IV corresponded to male larvae. To confirm this observation we obtained a sample of male larvae from a laboratory colony composed only by workers that had laid eggs and were raising the resulting males. It is worthy to point out that this observation was not previously reported for this species. Their head width was measured and mandibles observed and measurements corresponding to the more developed larvae matched exactly the head width and mandible type of larval instar IV.

The mandibles of larvae in each category of head width were examined and compared with the mandibles of male larvae mentioned before. Four types of mandibles were found, one of them exclusively on males. Thus indicating that the mandible is a good character to separate instars and can be used to recognize male larvae only in their

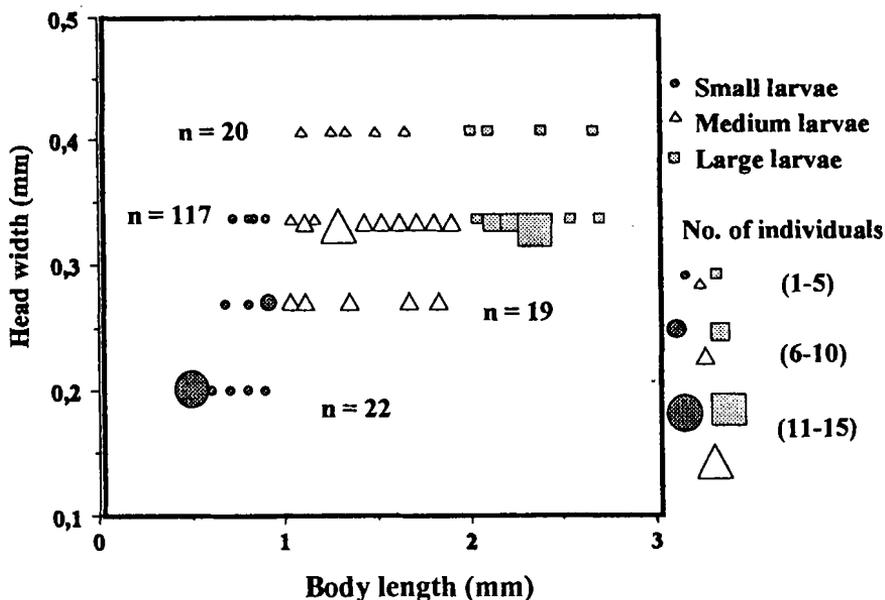


Fig. 2. Larval instars of workers and male ants of *P. fulva* as a function of head width and body length.

fourth instar, agreeing with the results reported by O'Neal & Markin (1975) and Ichinose (1987).

Total body length was also determined and ranged between 0.5-2.7mm, depending on the overall body size; larvae were grouped in one of three categories: Small (0.5-1.0mm), medium (1.0-2.0mm) and large (2.0-2.7mm).

Combining the information of both variables we could establish the existence of four groups of larvae, the fourth one corresponding to male larvae (Fig. 2). Finally we could determine the existence of three larval instars for workers and four for males, agreeing with the results obtained by Ichinose (1987) for *P. flavipes*, but differing in the fact that he reported four larval instars for the worker caste as well.

*Description of worker larvae.* The description of worker larvae was made employing some of the terminology used by Wheeler & Wheeler (1986) to depict other *Paratrechina* larvae. It is however a general description given with the practical purpose of being able to differentiate instars easily for other studies of developmental biology (Fig. 3).

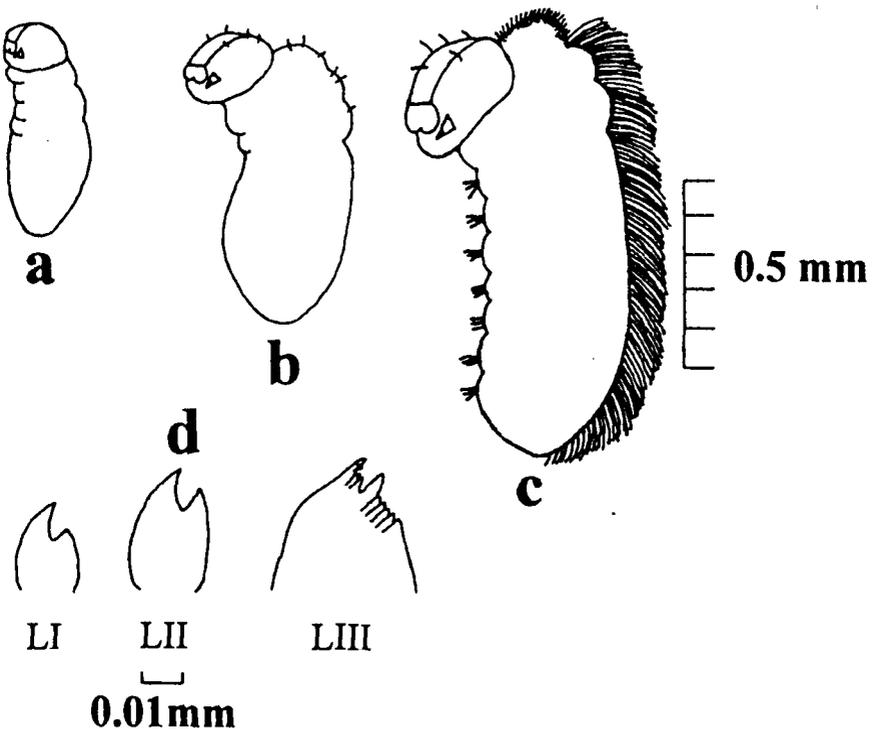


Fig. 3. *Paratrechina fulva*. Worker larvae. a. First instar larva. b. Second instar larva. c. Third instar larva. d. Mandible types. I-III instar larvae.

*First instar:* Small larvae. Lengths 0.5-0.9mm, mean 0.6 mm  $\pm$  0.1 S.D. Head width 0.20mm. Mandibles have two non-sclerotized teeth separated by a notch, the external one being almost two times larger than the internal. Head apical, abdomen straight with a round-pointed posterior end, body glabrous, its diameter almost uniform throughout.

*Second instar:* Small to medium larvae. Length 0.7-1.8mm, mean 1.0mm  $\pm$  0.3 S.D. Head width 0.27mm. Mandibles have two non-sclerotized teeth almost equal in length. Profile pheidoloid. Few body hairs localized on the dorsal surface of the head and thorax, sparsely distributed and some of them branched.

*Third instar:* Some small, but mostly medium to large larvae. Length 0.7-2.7mm, mean 1.7 mm  $\pm$  0.5 S.D. Head width 0.33mm. Mandibles have sclerotized teeth, seven small and two large; the external large tooth is bifurcated. Profile pheidoloid at an early stage, as they grow it becomes dolichoderoid. Large and abundant body hairs on the dorsal surface, uniformly distributed, some branched; few hairs on the ventral surface of each somite.

### **Description of male larvae**

Male larvae from instar I to III are almost identical in size and shape to the worker larvae, except for the tapering posterior end of their body and more distinctive somites. Larvae are illustrated (Fig. 4) but their description will not be given here.

*Fourth instar:* Medium to large larvae. Length 1.1-2.7mm, mean 1.8mm  $\pm$  0.4 S.D. Head width 0.4mm. Mandibles have sclerotized teeth, eight small and two large, the external large tooth is bifurcated. Profile dolichoderoid. Body hairs shorter than those of third instar larvae, abundant and uniformly distributed on the dorsal surface, some branched, whereas on the ventral surface of each somite only a few hairs. In general terms male larvae have less hair than worker larvae.

### **Immature development of the worker caste**

Within insects, the duration of the immature development changes with temperature among other factors. Under laboratory conditions (temperature 27°C, 80% R.H.), the development from egg to adult of *P. fulva* took from 23 to 50 days with a mean of 39.2  $\pm$  4.1 days. The average span of the immature development of *P. fulva* can be considered short compared with other members of the Formicinae group such as *Anoplolepis longipes* (59.6 days) (Rao & Veeresh 1991) another tropical CA; *Camponotus sp.* (48-74 days) (Mintzer 1979, as cited by Hölldobler & Wilson 1990) and *Prenolepis imparis* (70-90 days) (Tschinkel 1987 cited by Hölldobler & Wilson 1990) also have a longer immature development but these are not tropical species.

**Egg:** Eggs are spherical to oval, length  $0.39 \pm 0.02$  mm, width  $0.25$  mm  $\pm 0.01$ , slightly bigger than the size reported earlier by Zenner-Polania (1990 a), who worked with *P. fulva* from a different region of the country. Whitish color. Queens lay single eggs, which are stuck up by the workers with their saliva to form an egg mass containing  $17.9$  mm  $\pm 8.7$  S.D. eggs. Incubation time varied from 9 to 21 days ( $16.2$  days  $\pm 4.3$  S.D.).

**Larva:** Larvae are scattered by the workers on the moist surface of the artificial nest. They go through three instars, already described, and a prepupal stage (no moult takes place) in which they expel the meconium and stop feeding. This period takes from 7 to 15 days ( $10.8$  days  $\pm 3.0$  S.D.).

**Pupa:** Pupae exarate, length  $2.6$  mm  $\pm 0.2$  S.D., also slightly larger than the length reported by Zenner-Polania (1990a). Pupae are taken by the workers to dryer places in the nest, they are placed one over the other forming mounds. The color of the pupa is initially white and

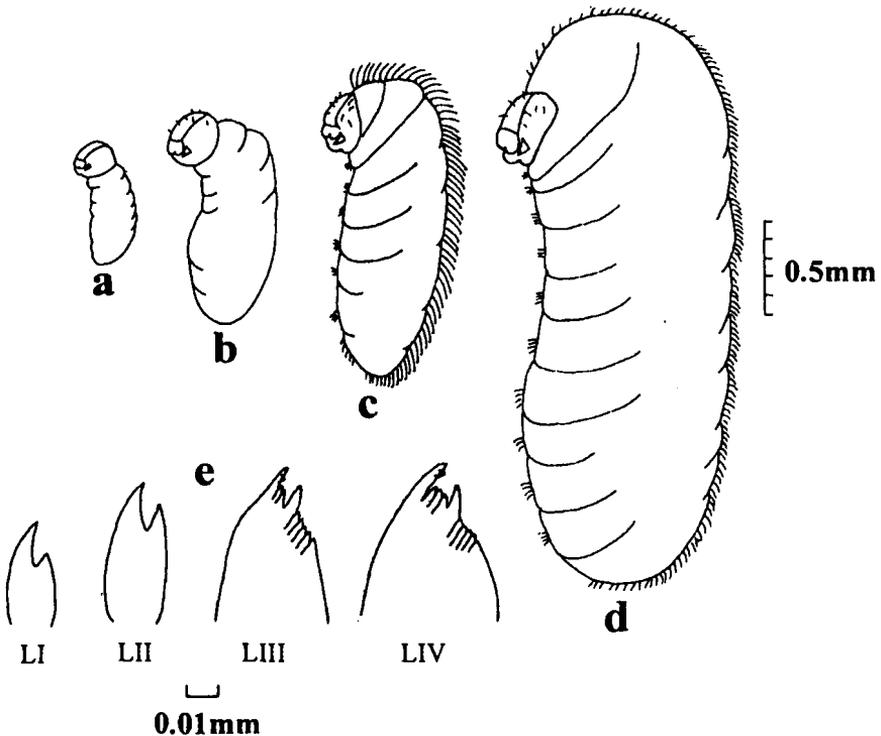


Fig. 4. *Paratrechina fulva*. Male larvae. a. First instar larva. b. Second instar larva. c. Third instar larva. d. Fourth instar larva. e. Mandible types. I-III instar larvae.

eventually changes to light brown. Duration of the pupal stage ranged from 7 to 14 days (12.2 days  $\pm$  3.0 S.D.).

### Colony growth under laboratory conditions

Monogynous, trigynous and hexagynous colonies were observed for a period of 10 to 11 weeks under laboratory conditions. The number of egg masses, larvae and pupae were recorded. We also calculated the pupae/eggs ratio as a way to quantify the development of the colony.

The highest production of brood was obtained in the hexagynous colonies followed by the trigynous and monogynous in decreasing order (Fig. 5). The maximum production of eggs in each type of colony was averaged; and we observed that it was reached faster in the polygynous colonies in contrast with the monogynous, although no significant difference was found; while the maximum number of larvae and pupae was obtained towards the end of the observation period for all of the colonies (Table 1). Regardless of the difference in incubation time, it seems that the first new workers should be produced at the same time for all of the colonies.

The greatest percentage of mortality was found during the incubation period and it reached an average of 70% approximately for the monogynous colonies; mortality was lower during larval development, close to 50% for the three kinds of colonies (Table 2). Significant differences were found between the mortality percentages of larvae ( $F = 5.18$ ;  $df = 6$ ,  $P < 0.05$ ), specifically between the monogynous and hexagynous colonies ( $q = 7.69$ ;  $df = 6$ ,  $P < 0.05$ ); in contrast we did not find significant differences among the mortality percentages of pupae for the three kinds of colonies.

Figure 6 illustrates the variation of the pupae/eggs ratio throughout development. The polygynous colonies reached and maintained faster a 0.5 limit for this ratio. As experimental units for future research, the polygynous colonies proved to be more stable, while the number of eggs per queen was greater in monogynous colonies (*unpublished results*); polygynous colonies showed a lower percentage of mortality of larvae

Table 1. Maximum number of eggs, larvae and pupae found in monogynous, trigynous and hexagynous colonies of *P. fulva* reared under laboratory conditions.

Kind of Colony	Maximum number of eggs	Day	Maximum number of larvae	Day	Maximum number of pupae	Day
Monogynous	138	44	62	53	38	61
Trigynous	270	21	92	46	70	68
Hexagynous	294	18	156	49	105	56

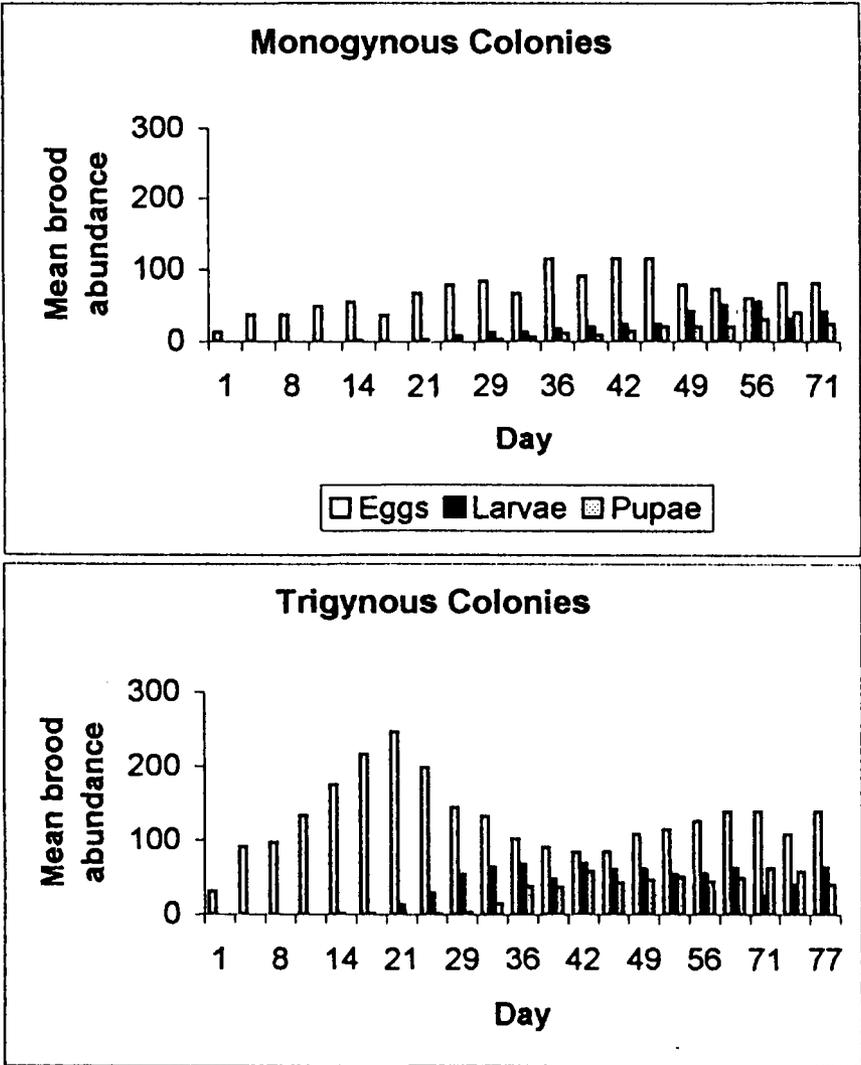


Fig. 5. See legend on facing page.

after the incubation period. Although no significant difference was found between the mortality percentages during development from larvae to pupae, polygynous colonies were more successful in rearing brood to the pupal stage, reaching and maintaining a pupae/eggs ratio of at least 0.5 faster than the monogynous colonies.

It has been demonstrated that worker number is correlated with egg

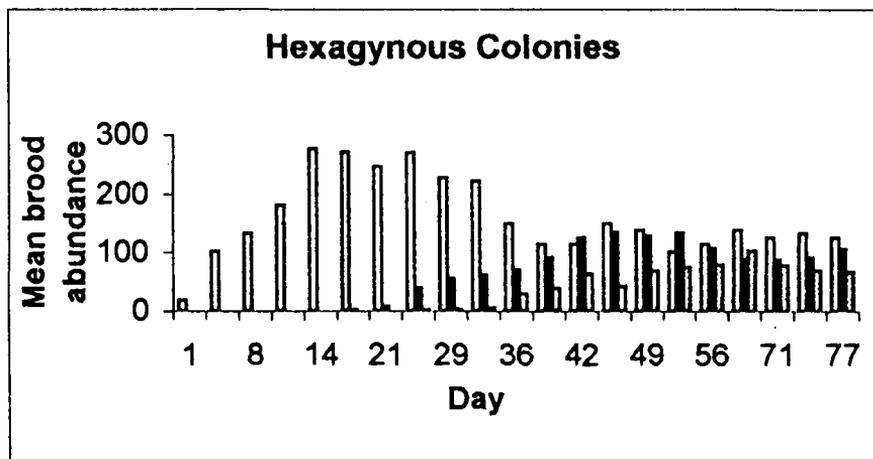


Fig. 5 (continued). Mean abundance of brood in monogynous, trigynous and hexagynous colonies of *P. fulva* reared under laboratory conditions.

Table 2. Mean mortality during incubation and larval development in monogynous, trigynous and hexagynous colonies of *P. fulva* reared under laboratory conditions. Means followed by the same letter do not differ statistically (Tukey test).

Kind of Colony	% Mortality during incubation	% Mortality during larval development
Monogynous	68 ( $\pm$ 7) (a)	52 ( $\pm$ 6)
Trigynous	63 ( $\pm$ 6) (a,b)	46 ( $\pm$ 1)
Hexagynous	49 ( $\pm$ 9) (b)	56 ( $\pm$ 3)

production and survival. A higher number of workers per queen yields a greater egg production up to a limit (Brian 1969, Passera 1972) but an adequate number of workers per queen needed for optimal egg production does not necessarily ensure an optimal brood survival (Brian 1983). Colonies of various sizes and species of *Myrmica* were studied by Elmes & Wardlaw (1981), all the species showed a similar significant regression between the number of workers and larvae but a higher workers/larvae ratio was observed in small colonies in contrast with larger colonies, thus probably indicating that larger colonies are more efficient in brood rearing than smaller ones. These results agree with our findings in that respect. Brian (1983) considers very crowded conditions to be responsible for inefficiency, many workers trying to get at the same few larvae may result in overlapping activities, redundancy, interference in communication, collisions, avoidance of impact with a consequent reduction of effective brood care.

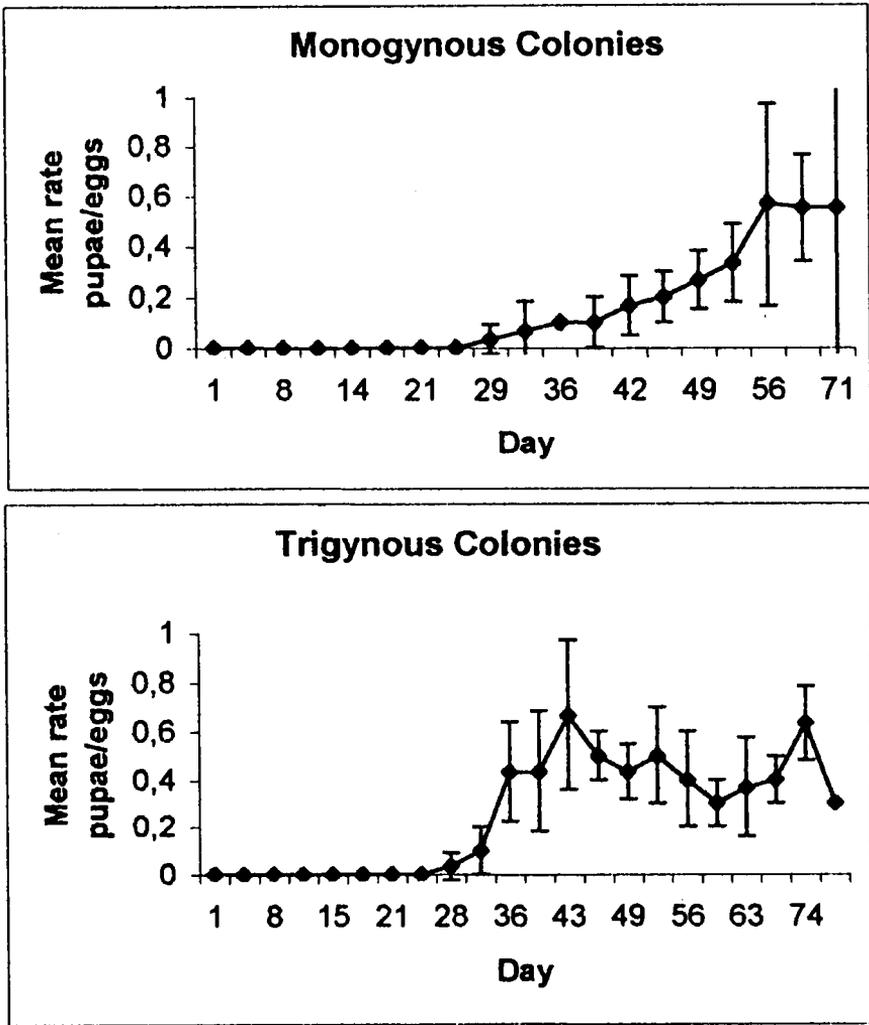


Fig. 6. See legend on facing page.

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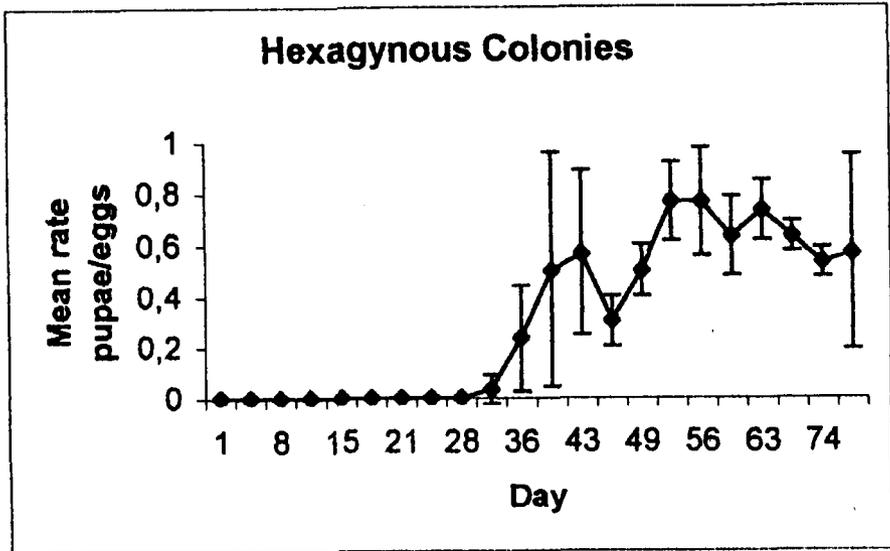


Fig. 6 (continued). Temporal variation of the mean rate pupae/eggs in monogynous, trigynous and hexagynous colonies of *P. fulva* reared under laboratory conditions.

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