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Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta**

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ABSTRACT. Social control of egg-laying rate in queens of the fire ant (*Solenopsis invicta* Buren) was studied by experimental manipulation of the number of larvae, pupae and workers in colonies, and the age and size of larvae and workers. Workers and pupae do not stimulate oviposition by queens. The number of fourth instar larvae, on the other hand, bears a positive log–log relationship to the queen's egg-laying rate. Such larvae are needed both to stimulate and maintain oviposition. Their withdrawal results, within 48 h, in a decline in queen oviposition almost to zero. Their addition to broodless nests results in peak laying in about 4 days. Larvae in the first three stadia and early in the fourth stadium have a much lower effect upon queen fecundity. Sexual larvae are only *c.* 5% as stimulating on a weight basis, but equivalent on an individual basis. Several associated measures are positively correlated to egg-laying rate: weight of the queen, the number of her vitellogenic follicles per ovariole, total vitellogenic follicles, the time she spends feeding and (usually) the number of workers in the retinue that cares for her. The egg volume is negatively correlated with laying rate, so that queens lay more eggs for the same expenditure of material as laying rate increases. As body size of workers increases, they become less effective in transmitting the larval stimulation to the queen, but worker age has no effect on this ability. For a given number of larvae, queens in small, naturally growing colonies lay fewer, larger eggs than do queens in experimental colonies, but their fecundity increases more rapidly in relation to number of larvae. When larvae are fed vital-dyed food in one experimental colony, and then transferred to an undyed colony, the dye is rapidly transferred to worker crops, and into the queen's eggs, indicating bulk movement of material from larvae to workers to the queen and eggs. Large larvae are more effective at this than small larvae. Fourth instar larvae may be a digestive and metabolic caste that processes protein for egg production by the queen. If that is the case, the queen and fourth instar larvae are linked in a positive feedback loop. Either the logarithmic relation of fecundity to larval numbers or physical limits of the

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queen may set the maximum egg-laying rate, and thus determine maximum colony size. The data do not allow a clear choice between these alternatives.

Key words. Formicidae, social insects, reproduction, fecundity, egg-laying, ovary, trophallaxis, workers, worker larvae, sex larvae.

Introduction

Strict reproductive division of labour is a hallmark of fully social insects. The colonies of many species of ants have a single queen who lays most or all of the eggs. The task of brood care is almost entirely carried out by the workers, who in turn contribute little or nothing to the egg pool. For maximum efficiency, this division of labour requires coupling between the queen's rate of oviposition and the availability of workers to care for the brood. For instance, it would be wasteful for a queen in a small colony to lay eggs at a rate exceeding the ability of workers to care for them. On the other hand, queens in large mature colonies must produce enough eggs to offset the typically high rates of worker mortality. In other words, colonies of social insects must regulate the oviposition rate of their queens. For species that develop large colonies, the range of oviposition rates must be enormous. The nature of this coupling or social control is of great interest, but most literature on the social control of oviposition rate focuses on worker oviposition and queen-control of worker reproduction.

The dominant hypothesis concerning the control of queen fecundity is that larvae have priority over queens in the competition for food and worker attention, making queen fecundity positively related to the number of workers and negatively to the number of larvae. A few studies have addressed this question, some supporting the hypothesis, others not. In *Plagiolepis pygamea*, the number of workers has a direct positive effect on queen oviposition (Passera, 1972). In *Myrmica ruginodis* and *M.rubra*, queen oviposition rate is saturated by as few as ten workers (Brian, 1953, 1957a, 1969; Mamsch, 1965, 1967). More workers do not bring about an increase in fecundity. Wilson (1974) found a weak relationship between the number of workers and the egg crop in *Leptothorax curvispinosus*. In *Odontomachus haematodes* (Colombel, 1970), an increase in queen fecundity parallels an increase in colony size and age.

With respect to a possible negative effect of larvae, Schneirla (1957) suggested that the synchronous metamorphosis of the larvae of *Eciton* caused workers to switch their feeding priority to the queen, and thus to initiate ovarian development and oviposition. Brian & Brian (1948) noted a similar association between queen oviposition and larval metamorphosis in *Myrmica* and bumblebees, and suggested a similar shift in worker attention. Queen–larval competition could explain the suppression of both queen and worker fecundity by high numbers of larvae in *Formica fusca* (Bier, 1954) and *M.ruginodis* (Mamsch, 1967). On the other hand, Brian (1957b) and Passera (1972) found no linkage between larvae and queen oviposition in *M.rubra* and *Plagiolepis*, respectively, and Evesham (1985) found evidence for queen–larvae competition only when the worker/larva ratio was low. When the supply of workers was adequate, no competition was detected.

Some of the above evidence is consistent with a negative feedback of larvae on queen, and perhaps worker, oviposition. Such feedback could be the origin of the cyclic egg production observed in several species, e.g. *Monomorium pharaonis* (Peacock & Baxter, 1950), *Myrmica rubra* (Brian, 1957a, b, 1965), *Leptothorax curvispinosus* (Wilson, 1974), young colonies of *Solenopsis invicta* (O'Neal & Markin, 1975; Markin *et al.*, 1972) and *Eciton burchelli* (Schneirla, 1957). Nevertheless, in *M.rubra*, a direct test indicated that the peaks in the standing crop of eggs were the result of oophagy by newly hatched larvae, not inhibition of queen oviposition by older larvae (Brian, 1957b).

Control of worker fecundity may also be relevant. In *Myrmica*, worker-laid eggs are proportional to the number of workers (Brian, 1969). Larvae, on the other hand, may have a more complex effect on the production of worker-laid eggs. Smeeton (1982) claimed that a small num-

ber of larvae stimulated trophic egg laying by workers but that large numbers inhibited such oviposition. Unfortunately, the differences were not consistently significant. The production of worker trophic eggs was stimulated by the queen (Brian & Rigby, 1978). In her absence, workers lay fewer eggs and these develop into males.

Two competing control mechanisms have been proposed for worker fecundity. First, there is suppression of worker fecundity by a queen-secreted pheromone, possibly as in the honeybee. Evidence of such a mechanism in ants is weak or absent (Carr, 1962; Brian & Hibble, 1963; Brian & Ribgy, 1978; Mamsch, 1967). Second, there is production by workers of a hypothetical 'profertile substance' sequestered preferentially by the queen and secondarily by the larvae (Bier, 1954). If queen and larvae are absent, this material builds up in the workers to induce their oviposition. Fecundity depends quantitatively on the ratio of producers (workers) and consumers (queens and larvae) (Mamsch, 1967). Passera (1972), Brian (1969) and Tschinkel & Howard (1978) showed that the per-queen fecundity decreases with number of queens per nest in *P.pygmaea, Myrmica rubra* and *S.invicta*, respectively, implying a constant amount of profertile factor. A possible profertile factor is the Juvenile Hormone (JH) which functions as a gonadotropin in adult insects, and initiates vitellogenesis in the primitively social bee, *Lasioglossum zephyrum* (Bell, 1973). Barker (1978) demonstrated that oocyte development and oviposition increase with JH dose in young fire ant queens. A number of Juvenile Hormone analogues (JHA) cause a reduction of oviposition in fire ants (Troisi & Riddiford, 1974; Banks *et al.*, 1978), a finding apparently inconsistent with a profertile role. However, these JHAs interfere with larval development, and the effect on oviposition may be a secondary result of the effect on larvae.

Obviously, a colony-founding queen of a species having a high potential fecundity does not start laying eggs with all of these mechanisms in gear. Rather, these are brought into play as the colony grows and the ovipositional demand increases. When all ovarioles are fully active at maximal rate, the queen has reached her maximum oviposition rate. Hermann & Blum (1965) reported that fire ant queens have eighty to ninety ovarioles per ovary. In newly mated queens, only the terminal follicle of four to six ovarioles is vitellogenic. The number increases to the terminal follicle of about twenty to twenty-five within 1 week (Hermann & Blum, 1965), but no data on further development are available.

Oviposition rate is basic to the population dynamics of colonies. Colony growth rate and maximum size depend, at least in part, upon oviposition rate. Colony size is itself an important evolved character of social insects, and its increase in monogynous societies must ultimately be accomplished through increased fecundity. Colony size among ant species ranges from a few dozen individuals to several million, implying an enormous range of fecundity rates among the queens (Tschinkel, 1987). We have only very sketchy knowledge of the morphological, physiological and behavioural adaptations associated with levels of fecundity and its control.

Present knowledge of the nature of population growth within a social insect colony is crude, but appears to fit a logistic curve (Bodenheimer, 1937; Wilson, 1971). This implies that density-dependent feedback or the emigration of sexuals gradually slows growth during the later stages of colony growth (Brian, 1965). Wilson (1971) has indicated that 'the identification and measurement of the factors comprising the feedback should become one of the principal targets of population studies in social insects, as it already is in the analysis of other animal species'. Certainly one of the most logical places to look for such feedback is the fecundity of the ant queen and its control by social factors. In this paper data are presented on the social regulation of queen fecundity in fire ant colonies.

Materials and Methods

A. *Collection and maintenance*

Queens and worker brood were collected in the field near Tallahassee, Florida, primarily during the winter and spring (Tschinkel & Howard, 1978). All colonies were of the monogynous form of *S.invicta*. Upon successful capture of the queen, large samples of workers and brood were also collected as the nucleus of laboratory stock colonies. General collection and handling procedure were as described by Banks *et al.* (1981).

Queens were maintained in stock colonies of

their own workers, housed in 150 mm plastic petri dishes with moist plaster floors. The dishes were set in foraging arenas consisting of plastic nursery or photographic trays whose sides had been painted with Fluon to prevent ant escape. Colonies were fed sugar water, beetle larvae and crickets, and watered as needed.

Queens from these stock colonies were used for experiments throughout the year. Between May and November, the experimental larvae were obtained from these stock colonies, and were thus laboratory-reared. Laboratory-reared larvae are less variable in size, an advantage for experiments in which larval age is estimated by larval size (see below).

B. *Basic experimental design*

Almost all important behavioural or life history characters vary among individual fire ant colonies. In order to separate this source of variation from that caused by the experimental factor(s), all experiments were set up in blocked designs. Workers from a single field colony (worker source colony) were divided into as many colony fragments (experimental colonies) as there were experimental treatments. Each experimental colony fragment was subjected to one of the treatments, so that each worker source colony was subjected to all treatments and formed one replicate set. In most experiments there was no within-block replication, but at least four replicate sets were run for each experiment.

A foreign queen was adopted into each experimental colony on the first day of the experiment. Fire ant colonies which have been queenless for at least 4 days readily adopt foreign queens. On the rare occasions when the adopted queen was attacked or killed, she was replaced with another. The queen from the worker source colony (if available) was never used in the same experiment as her workers. Worker brood of all stages was readily adopted across colonies, but adoption of sexual brood was inconsistent.

Workers and brood were usually allocated to experimental nests on the basis of live weight. Workers were separated from brood by etherizing lightly and spreading the ants out on construction paper. As the workers recovered, they clung to the paper but did not immediately pick up and hold the brood, so that these could be tipped off the paper. The clinging workers were shaken off into a separate container and the operation repeated until separation was complete.

Separation of pupae from larvae exploited the tendency of workers to segregate pupae from larvae in their nests. Rapid aspiration of pupal piles immediately after lifting the nest lid usually yielded mostly pupae. Final separation was carried out manually.

C. *Nest design*

Flat, rectangular experimental nests were cast from dental plaster ('Labstone', Columbus Dental, P.O. Box 620, St Louis, Mo.) and covered with a plate of glass such that ants were more or less one layer thick. Entrances were cut into the rim supporting the glass. An internal, U-shaped tunnel in the plaster base opened into two vertical glass tubes through which water could be applied to the nest. Nests of this design were made in a range of sizes for use with various numbers of workers and brood. The experimental nests were kept in plastic trays whose sides had been treated with Fluon to prevent escape of workers. Food was provided *ad libitum* in most experiments in this foraging arena, and the nests were watered as needed. Colonies were maintained and all experiments run at 30°C.

D. *Dependent variables and their measurement*

In most experiments the experimental nests were placed under a dissecting microscope which was moveable in a horizontal plane, allowing tracking of the ants as needed. The following measurements were made in almost all experiments: rate of oviposition, size of queen's retinue, time queen spent feeding, gaster length of queen, weight of queen, number of larvae, pupae and eggs, and stage of ovarian development.

Oviposition rate was determined by direct observation for 30 min periods and recorded on an event recorder. Both timing and rate of oviposition could be determined. Reliability of the counts was very high because the workers in the circle attending the queen (her retinue) showed distinctive behaviour when they moved forward to pick an egg from the queen's genital aperture. Having grasped the egg between their mandibles, they backed out to the margin of the

retinue where they paused briefly, allowing verification of the egg. They then left the queen to add the egg to an existing egg-pile. The primary source of confusion was egg-carrying workers who did not immediately leave the vicinity of the queen, or who returned. In nests with actively laying queens, this was not commonly observed.

The pattern of oviposition in time affects the sampling characteristics. Batch-wise or clumped egg-laying would probably require longer observation periods because of increased variance. To assess temporal patterns, number of eggs laid in each of twelve equal increments of the 30 min observation period of eight queens laying at moderate to high rates on three consecutive days were determined. Dispersion of eggs among these twelve increments was compared to the Poisson distribution by computing the variance/mean ratio, and comparing the significance of its deviation from 1 (random) by means of the χ^2 value. In fifteen cases the dispersion did not differ significantly from random, but in nine it was significantly uniform. Sixteen other queens laying at lower rates dispersed their eggs randomly in time. A single queen showed a clumped dispersion. Altogether, these patterns indicate that any 30 min period is a reasonable sample of egg laying.

Queen retinue size was determined by three rapid counts of the circle of workers orientated with their heads towards the queen or showing other signs of attention toward the queen. The midpoint of these counts was used for analysis. Workers in the retinue groom the queen, feed her (through trophallaxis), carry away the eggs and imbibe the large droplets of liquid faecal material she produces.

Queen feeding by trophallaxis with workers was recorded by depressing an event recorder

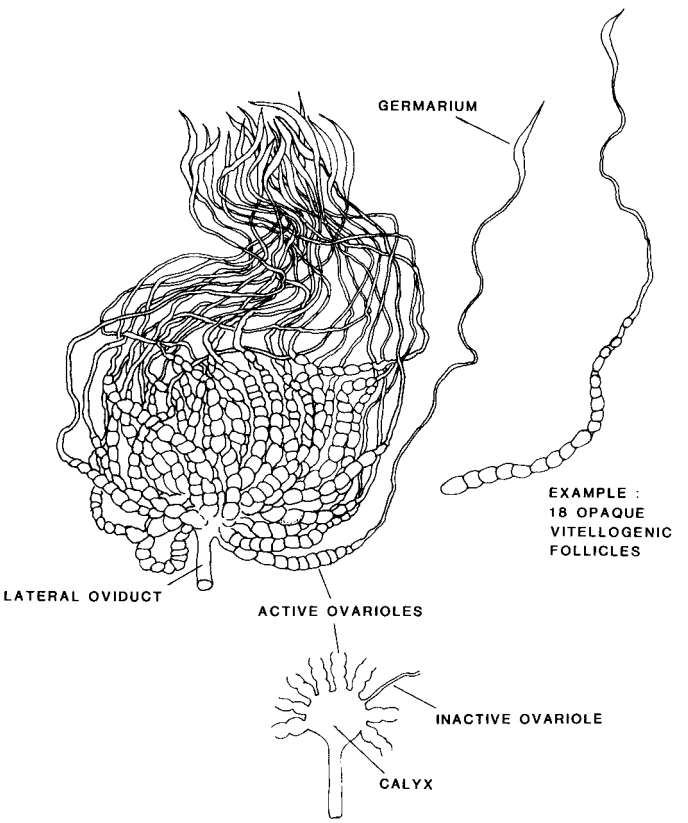

FIG. 1. Active ovary of a fire ant queen, showing how ovarian activity was estimated. The number of vitellogenic (opaque) follicles was counted in ten haphazardly selected ovarioles, averaged and multiplied by the number of active ovarioles to give the total vitellogenic follicles in the ovary. Number of inactive ovarioles was also counted. Saggital section at bottom shows arrangement of ovarioles around calyx. Meiosis takes place in germarium.

key as long as the queen's mouthparts remained in contact with those of a worker. Total time (minutes) spent feeding during each 30 min observation period was analysed for all feeding events longer than 5 s.

The queen's gaster length was recorded in arbitrary units using an ocular micrometer. By calibrating the live weights of queens to their gaster length, queen weight could be estimated without intrusion during an experiment. Correlation of gaster length to live weight was high (R^2=0.72).

Queens were weighed at the time of set-up and termination of each experiment. Queen weight varied primarily as a result of ovarian development, but also in relation to fullness of the crop.

Estimates of life stages were made by rapid counts, using a tally-counter. Stages usually distinguished were feeding larvae (pink to dark coloured), pharate pupae (white larvae), pupae and eggs. Duplicate counts were usually reproducible to within 10–20%.

Estimation of ovarian development followed dissection of the ovaries at the termination of most of the later experiments. Ovaries and the remainder of the queen were stored in 2.5% glutaraldehyde in buffered insect Ringer solution. Each ovary (Fig. 1) was separated into five to ten clusters of ovarioles to ease a count of the number of active ovarioles (those with opaque, vitellogenic follicles) and inactive ovarioles (lacking opaque follicles, and usually thin and threadlike).

(a) The number of vitellogenic follicles in ten haphazardly selected active ovarioles was counted and the counts averaged. The product of the average number of vitellogenic follicles per oviariole and the number of active ovarioles then gave an estimate of the total number of vitellogenic follicles in the ovary. The sum of the two ovaries gave the total for the queen.

(b) All actively laying queens contain ovulated eggs in their ovarian calyx. The length and width of these eggs were measured using a calibrated ocular micrometer and their volume was computed as the volume of a prolate spheroid. The mean volume of up to ten eggs per queen was used for analysis.

E. *Analysis of data*

Most results were analysed as two-way analysis of variance (ANOVA) in which worker source colony was a blocking (random) factor and treatment, sometimes with multiple levels, the other factor. In a few cases, three-way ANOVA was used because replicate sets could not all be run simultaneously. Time or larval source then formed a second blocking factor. All statistics are from ANOVA unless otherwise noted. Most were available in Minitab programs (Ryan *et al.*, 1982).

Experiments and Results

Rationale. Because of their greater ovarian development, queens captured from large colonies were generally heavier than those from small colonies. This implied that social factors related to colony size might be related to fecundity. I elected to test number of workers, pupae and larvae independently, because together these determine colony size. For those categories that showed an effect on queen fecundity, I tested certain characteristics such as size and age.

Worker number. Broodless experimental colonies containing 750, 1500, 3000 or 6000 workers were set up in observation nests of proportional area. Each colony contained its own queen and the series was replicated four times, making the experimental design a completely random one, suitable for one-way ANOVA. The egg laying rate of the queens in these nests after 4 days was not significantly related to worker number and was low in all treatments (one-way ANOVA, F=1.50; df=3, 12; N.S.). Workers in broodless nests tended to pack tightly into one part of the nests, often with the queen in their midst, without a retinue. Body weight, gaster length and ovarian development of such queens were all low, reinforcing the picture of low egg-laying rates. Thus, workers alone are unable to stimulate or sustain oviposition in queens.

Number of larvae. A preliminary experiment had shown that larvae added to broodless colonies stimulated a burst of oviposition by queens beginning 1 day after larval addition. Larvae were thus tested in combination with worker number as follows: each of two worker source colonies was divided into six fragments, three containing 1500 and three containing 6000 workers. A randomly selected queen from a laboratory colony was then adopted into each

experimental colony. Each level of workers received either 0, 1500 or 6000 field collected larvae of medium to large size. Larvae without workers were not tested. The experiment was thus a three-factor design with two replicates, in which worker source colony represents a blocking factor.

Queen oviposition rate 4 days after addition of larvae was directly related to the number of larvae (ANOVA, $F=32.2$; df=2, 9; $P \ll 0.001$), but independent of the number of workers ($F=1.00$; df=1, 10; N.S.). Oviposition rate differed between all levels of larval number: 3, 9 and 17 eggs/30 min for 0, 1500 and 6000 larvae, respectively (Duncan's test, $P<0.01$), but did not differ significantly between levels of worker number within larval treatments. It thus appears that larvae stimulate and sustain queen oviposition, but that between larva/worker ratios of 1:4 and 4:1, the number of workers is of no consequence. Queen body weights, as estimated from gaster lengths, increased in relation to larval numbers but fell just short of significance.

The relationship between number of larvae and oviposition rate appeared to be non-linear and was further tested in an experiment using a wide range of larval numbers. Each worker source colony was divided into five experimental colonies of about 3000 workers (3 g). A randomly selected queen from a laboratory stock colony was adopted into each experimental colony, and each colony received either 1, 10, 100, 1000 or 10,000 large larvae (estimated by weight). The entire experiment was replicated four times over a period of 3 weeks. Four days after the larvae were added, the queen's oviposition rate and other measures were determined and the replicate terminated.

The queen's egg laying rate is strongly dependent upon the number of larvae (Fig. 2). ANOVA on the log–log transformed data showed that the log number of larvae explained 94% of the variation in the log of the egg-laying rate ($F=59.3$; df=4, 12; $P \ll 0.001$). The mean values increase from 1.3 eggs per 30 min with one larva to 37 with 10,000 larvae.

As the number of larvae increases, additional larvae result in ever smaller increments in egg-laying rate (Fig. 2). Thus eggs per 30 min/larva declined about 430-fold from 1.6 to 0.0037 over the tested range of larval numbers. ANOVA on the log–log transformed data showed that the log larval number explained 97% of the variation in the log egg-rate/larva ($F=172$; df=4, 12; $P \ll 0.001$).

Eggs are significantly smaller at high egg-laying rates ($F=5.03$; df=4, 12; $P<0.05$) (Fig. 3). In Fig. 3 the volume of material (eggs per 30 min×egg volume) the queen would oviposit if egg volume were constant at its maximum value in this experiment is shown as 'projected total'. The actual volume at the highest oviposition rate is 22% lower than the projected, indicating that the queen oviposits 22% more eggs for the same amount of material by laying

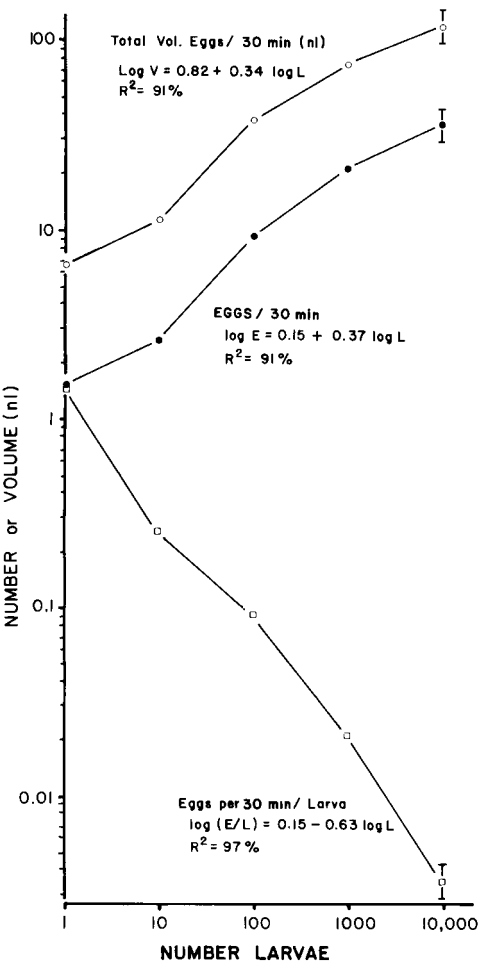

FIG. 2. The egg-laying rate, total volume of material oviposited and egg-laying rate per larva in relation to the number of fourth instar larvae. Increasing larval numbers causes both egg-laying rate and total volume eggs to increase and egg-rate per larva to decrease. Error bars=±1 SEM from pooled ANOVA data, shown for one point only.

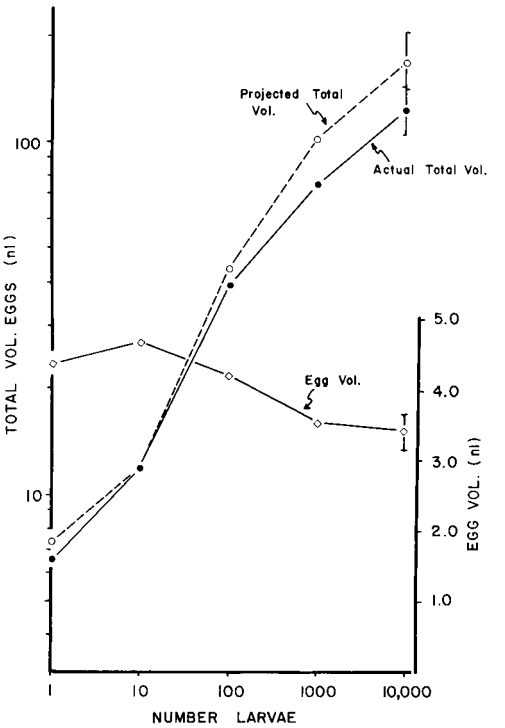

FIG. 3. If egg volume were to remain constant at its maximum value in this experiment with increasing egg-laying rate, the total volume of eggs produced by the queen would be much higher (dotted line) than the observed (solid line). Queens are able to lay more eggs with the same output of material by laying smaller eggs at higher rates. Error bars=±1 SEM from pooled ANOVA data, shown for one point only.

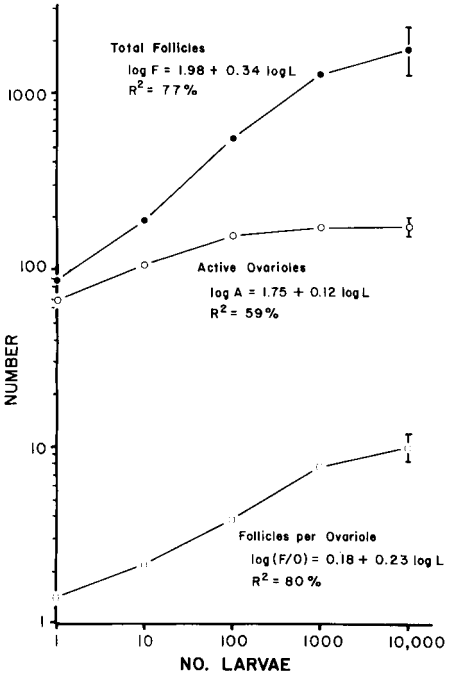

FIG. 4. Three measures of ovarian activity as a function of the number of fourth instar larvae (log scale). Because essentially all ovarioles are activated by only 100 larvae, the increase in total follicles when larval number exceeds 100 is primarily through an increase in the number of vitellogenic follicles per ovariole. Error bars=±1 SEM from pooled ANOVA data, shown for one point only.

smaller eggs. Nevertheless, the log number of larvae still explains 94% of the variation in the log total volume of material laid in eggs (F=53.7; df=4, 12; $P \ll 0.001$).

Ovarian hypertrophy accompanies the increased oviposition rate. This is reflected in the significant increase in queen weight in relation to the log number of larvae (Fig. 5; ANOVA, F=23.8; df=4, 12; $P \ll 0.001$). While some of this increase may be the result of more food in the crop, most is undoubtedly the result of the increase in ovarian development (Fig. 4). As number of larvae increases, at first fecundity is increased by activating more ovarioles, but it only requires about 100 larvae to activate all ovarioles. At the same time there is a steady increase in the number of vitellogenic follicles per active ovariole, so that the total number of vitellogenic follicles increases enormously over the tested range of larval numbers (Fig. 4). ANOVA of the log–log transformed data showed that log number larvae explained 80% of the variation in log total vitellogenic follicles (F=18.2; df=4, 12; P<0.001), 82% of the variation in vitellogenic follicles per ovariole (F=25.1; df=4, 12; P<0.001) and 71% of the fraction of ovarioles active (F=10.2; df=4. 12; P<0.002; only number of larvae was log transformed for this last analysis).

What is the exact nature of the relationship between the queen's fecundity and the number of larvae? Two-way linear regressions were computed for the untransformed data, again after log-transformation of the number of larvae only, and both variables. Log–log plots still showed sigmoidality (Figs. 2–4), so the log of each dependent variable was also normal-score transformed (Ryan *et al.*, 1982). The Lack of Fit test (Draper & Smith, 1981) and analysis of residuals

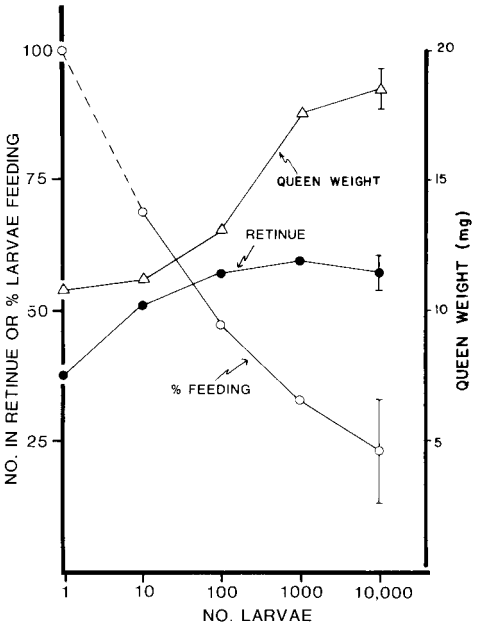

FIG. 5. The number of workers in the queen retinue, final queen weight, and the rate of larval development (measured as per cent larvae still feeding at end of experiment) as a function of the number of fourth instar larvae. Retinue size increases to a plateau and larvae develop more rapidly as the number of larvae increases. Error bars = ±1 SEM from pooled ANOVA data, shown for one point only.

were applied to each of these regressions. For four of the five dependent measures (eggs per 30 min, total follicles, total volume eggs, follicles per ovariole) only the untransformed data showed significant lack of fit to linearity ($P<0.05$ to 0.001). For eggs per 30 min/larva, the semi-log transformed regression was also significantly non-linear ($P<0.02$). For all five dependent measures, however, the log–log transformed regressions explained the highest fraction of the variation (80–97%) and showed the least pattern in residual analysis.

The best linear fit for these five dependent measures thus seems to be a logarithmic one. This means that each (y) is related to the number of larvae (x) as a power function. In its log form, this is $\log y = b + a \log x$. This relationship is similar to that of two body parts growing at constant differential rates, and means that as the larval population grows, the egg-laying rate, total volume eggs, egg-rate per larva, follicles per ovariole and total follicles all grow at a constant relative rate. For example, for every 10-fold increase in number of larvae, the egg-laying rate increases by a factor of about 2.3, the total volume of eggs by 2.2, the total follicles by 2.2 and the follicles per ovariole by 1.7. The ratio of these multipliers to the larval multiplier is approximately constant over the larval range. Because the multipliers of the dependent variables are only 17–23% as large as the multiplier of the number of larvae, all measures of fecundity per larva (=efficiency) decline with increasing larval numbers (Fig. 2).

The regressions confirm a laboratory observation, namely, fecundity is never zero, even in the absence of larvae (Figs. 2 and 4). With fewer than one larva (approximately zero), the queen still lays about 1.3 eggs per 30 min, contains about ninety-five vitellogenic follicles, or about 1.5 follicles in each of the approximately sixty active ovarioles. Thus, a queen without larvae is able to produce a few and so to build up fecundity as the larval population grows.

At the upper end, a question of interest is whether the upper limit of fecundity is set by the physical capacity of the queen or by the ability of the colony to achieve the enormous larval numbers needed to increase fecundity further. With respect to number of larvae, even at peak season, mature colonies of *S.invicta* rarely contain more than 15–20% larvae (Markin & Dillier, 1971), perhaps 15,000–30,000 larvae. This may be the result of declining efficiency of brood rearing by workers (Porter & Tschinkel, 1985b). Translated into eggs per 30 min, this would indicate a mean upper limit of fifty to sixty-four eggs per 30 min, respectively. There is considerable variation, however, and queens freshly captured in the field had rates as high as ninety-nine eggs per 30 min! In addition, field queens operate at unknown temperatures.

The alternate limiting mechanism would be suggested if fertility levelled off at the highest larval numbers indicating limits in the queen's physical ability to respond. The hint of plateau seen in Figs. 2 and 3, however, does not cause significant deviation from linearity, nor are the uppermost points significantly below the values predicted by the regression. Experimental treatments with more than 10,000 larvae are required to resolve the question of upper limits, but such experiments were not done. At this time, it is not possible to choose with confidence between these two alternatives.

TABLE 1. Pairwise linear regressions of experimental variables using pooled data from all experiments except worker number, worker age and pupa number. Coefficients are tabulated for the equation $y=b+ax$. SD=standard deviation of y about the regression; R^2=correlation coefficient; df=degrees of freedom; F-ratio=regression mean squares/error mean square; P=P-value from F-tables. The P-values were adjusted for multiple tests through the Bonferroni procedure.

y (units)	x (units)	b	a	SD	R^2 (%)	df	F-ratio	P-value
Follicles/ovariole	Eggs/30 min	2.07	0.255	2.78	65.2	78	149	≪0.001
Retinue	Eggs/30 min	43.98	−0.026	11.3	0.10	85	0.1	>0.5 N.S.
Total follicles	Eggs/30 min	224	52.9	501	71.3	78	197	≪0.001
Egg volume (nl)	Eggs/30 min	4.38	−0.021	0.546	25.3	70	25.1	<0.005
Time feeding (min)	Eggs/30 min	2.44	0.209	5.25	14.8	94	17.5	<0.005
Total follicles	Follicles/ovariole	−66.9	188	309	89.1	78	646	≪0.001
Egg volume (nl)	Follicles/ovariole	4.53	−0.078	0.51	34.4	70	38.4	≪0.001
Egg volume (nl)	Total follicles	4.50	−0.004	0.501	37.1	70	42.8	≪0.001
Eggs/30 min	Queen weight (mg)	−34.9	3.43	10.1	53.6	98	115	≪0.001
Total follicles	Queen weight (mg)	−2957	266	500	71.4	78	198	≪0.001
Follicles/ovariole	Queen weight (mg)	−13.0	1.27	2.82	64.0	78	142	≪0.001
Egg volume (nl)	Queen weight (mg)	5.52	−0.096	0.573	17.5	70	16.1	<0.005
Retinue	Queen weight (mg)	35.5	0.505	11.2	0.90	85	1.78	>0.2 N.S.
Time feeding (min)	Queen weight (mg)	−6.55	0.815	5.21	16.2	94	19.4	<0.005

Because the egg-rate, total follicles, follicles per ovariole and total volume eggs are all related to the number of larvae in a similar fashion, they are also correlated to one another (Table 1). Thus, every increase of one egg per 30 min in the egg-laying rate is the result of an additional fifty-three vitellogenic follicles, or 0.26 follicles per ovariole. Additional correlations are discussed below.

It is possible that egg-laying rate is increased by more rapid movement of eggs down the ovarioles. If the rate of yolk deposition remains constant, this would simultaneously account for the decrease in egg volume. The development time for ova from the time they are first detectably opaque until they are laid was estimated by dividing the total number of vitellogenic follicles by the egg-laying rate in eggs/h to give the hours it takes for an egg to develop. In relation to larva number, this time decreases by about 26% corresponding well to the egg volume decrease of 22%. The development time was variable and not significantly related to larva number, but declined from 41 h to 25 h as larva number increased from one to 10,000.

The size of the queen's retinue also increased with larval number (F=6.00; df=4, 12; P<0.02) (Fig. 5). Most of the increase took place between one and 100 larvae. The function of the larger retinue is probably to service the queen's greater feeding needs and to carry away the increased number of eggs. Available space around the queen must ultimately limit retinue size, but my impression is that the highest mean sizes are not yet at this limit. Retinues are dynamic, and workers enter and leave them constantly so that their number necessarily fluctuates rapidly around a mean value.

Two measures showed no significant relation to larval numbers. These were initial queen weight and total number of ovarioles.

Of the brood added to the experimental nests, an average of 92% could be accounted for on day 4, but only a mean of 24% of these were still feeding larvae (Fig. 5). The remaining 76% had transformed into pharate pupae or pupae. If the nests receving one larva are eliminated from the analysis because of small sample size, there is a significant inverse relationship between the fraction of larvae still feeding and the number of larvae in the nest (F=10.9; df=3, 10; P<0.005). Larvae appear to pupate earlier in nests with high larva-to-worker ratios, perhaps as a result of the shortage of worker care. Earlier pupation may result in smaller mean pupal size and smaller size variation, as reported by Porter & Tschinkel (1985a) in experiments relating pupal size to worker-to-larva ratios.

Rate of rise and fall of queen fecundity. If larvae are added to broodless nests, the egg-laying rate of the queen rises rapidly. The time course of this activation was determined in an experiment in which experimental colonies were read and killed 0, 2, 4 and 6 days after addition of larvae. Each worker source colony yielded four experimental colonies with 1.5 g workers each.

The queens were primed for 1 week in broodless fragments of the source colony in which they were to be tested. On day 0 of the experiment a primed queen and 1.5 g of large larva were transferred to each of the four experimental nests, one of which was read and terminated immediately as the Day 0 sample. On Day 1 of the experiment the number of pharate pupae and pupae were counted and an equal number of feeding larvae added to each nest. On Day 2 another queen was read and terminated and the brood in the remaining two nests were removed and replaced with fresh feeding larvae. On Day 3, non-feeding stages were once more counted and an equivalent number of feeding larvae added. This was followed by a queen reading and termination on Day 4, with the remaining nest receiving fresh larvae, addition of larvae on Day 5 and reading and termination on Day 6. this experiment was replicated simultaneously with five worker source colonies.

Because a different queen was used for each timed sample, the data for the 4 days are completely independent rather than a repeated measure, and thus amenable to ordinary two-way ANOVA. The effect of the larval replacements and additions was to keep the number of feeding larvae as constant as possible, both through time and across replicates. Even so, there were significant ($P<0.001$) differences between the number of feeding larvae in Day 2 and 4 nests ($\bar{x}=1600, 800$, respectively), underlining the difficulty of fixing these parameters.

The egg-laying rate was significantly related to time ($F=15.6$; df=2, 8; $P<0.01$), rising from 3.2 eggs/30 min on Day 0 to 16.4 eggs/30 min on Day 4 (Fig. 6). This increase is the result of a significant increase in the number of active ovarioles ($F=19.6$; df=2, 6; $P<0.005$) and the number of vitellogenic oocytes per ovariole, so that the total number of vitellogenic oocytes increased significantly ($F=36.3$; df=2, 6; $P<0.001$) from 164 to 1093 (Fig. 6). Assuming that the stimulative larval factor is either present or appears much more rapidly than queen fertility, Fig. 6 shows the rate at which queen fecundity increases under conditions of more or less constant number of larvae. It is probably a reasonable estimate of the maximum rate of fertility increase of which the queen is capable. Throughout this experiment, variation in the number of feeding larvae explained 73% of the variation in the egg-laying rate (regression on log-transformed data: $R^2=73.3\%$; $F=39.8$; df=1, 14; $P<0.001$).

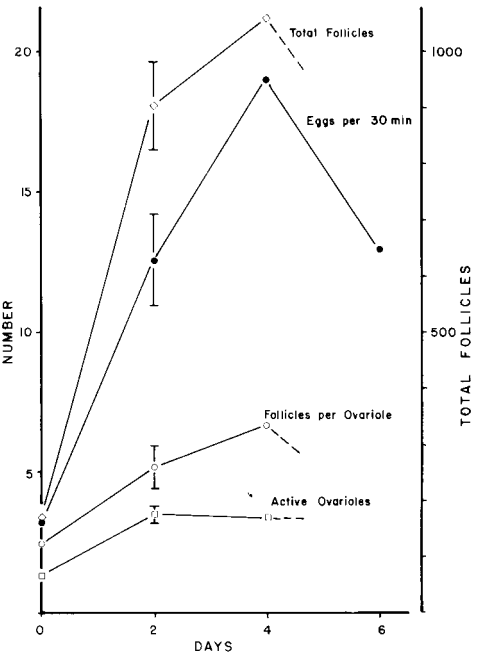

FIG. 6. Queen egg-laying rate and ovarian activity as a function of time after addition of larvae to broodless nests. Oviposition rises until day 4 and then drops. Error bars=±1 SEM from pooled ANOVA data, shown for one point only.

The egg-rate on Day 6 dropped to 9.6 eggs/30 min even though the number of feeding larvae was kept as constant as possible. Queens were not dissected on Day 6.

A variant of this experiment yielded similar results. Rather than priming queens in broodless colony fragments, the primer fragments contained 0.15 g of larvae. Rather than replacing and adding larvae throughout the experiment, each experimental colony received 1.5 g large larvae on Day 0 only. As larvae transformed to pharate pupae and pupae, they were not replaced, so that by Day 6 there were very few feeding larvae left.

Under these conditions, fertility throughout the experiment was shifted to higher levels, but the temporal pattern was similar, rising to a maximum at 4 days and then dropping off. Because larvae which pupated were not replaced, the drop in fertility on Day 6 could be due to the pupation of most of the larvae, but this is contradicted by the occurrence of a similar

drop even when larvae were replaced by Day 6. Probably, control of fertility is more complex, and may depend on the size as well as the number of larvae present (see below).

Removal of larvae brings on a rapid drop in queen fecundity. For this experiment, queens were captured in the field, and set up in broodless observation nests with about 250 of their own workers no later than 90 min after capture. All measures were taken at about 5, 24, 30 and 48 h after capture, and the experiment was replicated three times, with a total of twelve queens.

All measures of fecundity plummet almost to zero within 48 h after removal of larvae. Egg-laying rate, vitellogenic follicles per ovariole and total vitellogenic follicles all show highly significant declines (Fig. 7; $F=10.3$; $P<0.02$; $F=203$; $P<0.001$; $F=68.9$; $P<0.001$, respectively; all with df=3, 6). At the same time, the queen's weight dropped almost 10 mg from a mean of 23.9 mg (SD=3.4 mg) to 14.3 mg (SD=0.85 mg), a loss of 40% of the original weight

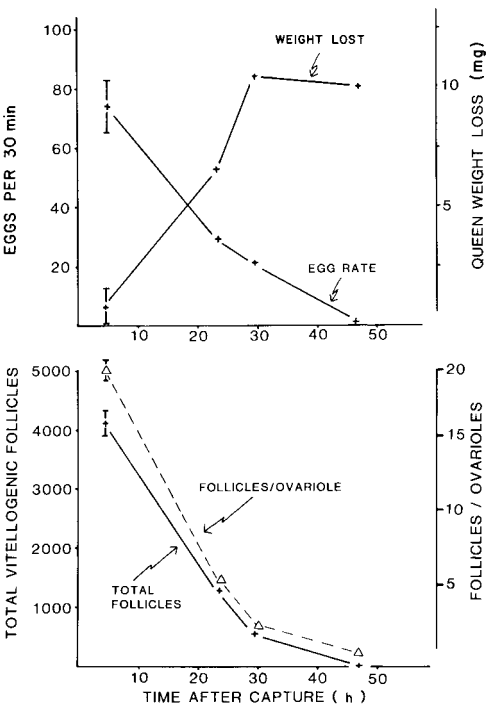

FIG. 7. Egg-laying rate and ovarian activity after withdrawal of larvae from field queens. All measures of fecundity decline almost to zero within 48 h. Queens lose *c.* 10 mg body weight in the form of oviposited eggs. Error bars=±1 SEM from pooled ANOVA data, shown for one point only.

($F=38.2$; df=3, 6; $P<0.001$). Most of this is probably lost as oviposited eggs.

This experiment demonstrates dramatically that larvae are required to sustain as well as initiate queen fecundity. Withdrawal of larvae from large colonies results in a more gradual decline of fecundity, perhaps because the workers act as a reservoir for the larval factor, but this was not tested experimentally.

Larval size or age. Fire ant larvae develop through four stadia, with most of the growth occurring in the fourth stadium. It is likely that the fecundity stimulating ability of larvae varies with size and stadium (or age). Laboratory-reared larvae were separated by size by placing them on a standard geological sieve and tapping the sieve lightly. Large larvae were retained on a U.S. Standard Testing Sieve No. 20 and consisted almost exclusively of late fourth instar. Small larvae were those which passed through a No. 25 sieve and consisted mostly of early fourth and third instars. First and most second instar larvae cluster with the eggs and mostly stuck to the walls of the aspirators during separation.

The effect of larval size (confounded with age) was tested with a constant live weight of larvae, as follows. Each worker source colony yielded three experimental colonies of 1.5 g workers each, and each was allowed to adopt a queen from a stock laboratory colony at the beginning of the experiment. At the same time, one colony received 1 g large larvae, the second 1 g small larvae, and the third 0.5 g large and 0.5 g small larvae. After 4 days the queens were read, dissected and all measures taken. Because small larvae were in limited supply, this experiment was run with two worker source colonies at a time, on four separate dates. Because larval batches were not identical on separate dates, larval batch is thus an additional factor (four replicates). Two worker source colonies are nested within each larval batch. The ANOVA was run accordingly.

Small larvae have a much lower ability to stimulate and maintain queen egg-rate than do large larvae ($F=10.2$; df=2, 14; $P<0.005$) (Fig. 8A). The egg-laying rate of queens with large larvae was nearly 3 times that of queens with small. The mixture was halfway between.

With respect to ovarian development, the total number of vitellogenic follicles followed a similar pattern (Fig. 8B) ($F=6.57$; df=2, 12; $P<0.05$). The same was true for vitellogenic

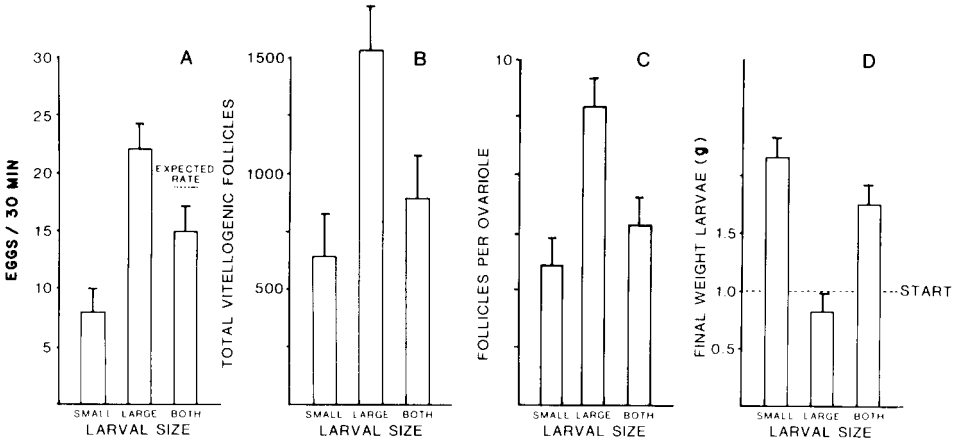

FIG. 8. (A–C) Queen egg-laying rate and ovarian activity in response to equal weights of large larvae, small larvae or both in 1:1 ratio. Small larvae are much less stimulating to oviposition than large ones. The mixture is intermediate, but lower than expected (see text). (D) Final total weight of larvae at end of experiment. Large larvae show no significant weight increase. Error bars=±1 SEM from pooled ANOVA data.

follicles per ovariole (F=10.4; df=2, 12; P<0.005) (Fig. 8C).

Larval batch (two replicates per batch) had a significant effect on ovarian development (P<0.01), indicating that my ability to select exactly equivalent larvae from batch to batch was quite limited, and that these batch-effects can be quite large.

It appears that the larval stage which most stimulates oviposition is the late fourth instar. Of the large treatment larvae, 77% were in post-feeding stages by the end of the experiment, while only 28% of the small larvae had similarly transformed. It is possible that the stimulation of fertility occurs as a single event at the end of the larval stage, around the time of larval–pupal apolysis. At this time, it is not possible to pinpoint the effect more closely.

The total live weight of larvae at the end of the experiment showed the opposite pattern: weight of larvae was highest in the small-larva treatments, lowest in the large, and intermediate in the mixture (F=19.6; df=2, 14; P<0.001) (Fig. 8D). The small larvae more than doubled their weight, the large lost about 20% and the mixed gained about 75%.

The degree of stimulation provided by large and small larvae together is of interest. Assuming no contribution by small larvae, the relationship between oviposition rate and number of large larvae (Fig. 2) was used to predict that the oviposition rate in mixed treatments, which contained 500 large larvae, should have been 19.0 eggs/30 min. The observed rate in mixed colonies was significantly lower at 14.3 eggs/30 min (T-test, pooled SD=5.70; df=10; P<0.01) and is the rate expected of about 220 large larvae. This implied that small larvae may actually inhibit the oviposition stimulation by large larvae by over 50%. The total follicles in queens with 500 large larvae (Fig. 4) was significantly higher than when mixed with 500 small larvae (T-test, P<0.001; pooled SD=477). A further experiment did not substantiate any inhibitory effect of small larvae upon large. Treatments consisted of either 0.5 g large larvae, 2.0 g large larvae or a mixture of 0.5 g large and 1.5 g small larvae. Inhibition by small larvae should have depressed the fecundity of queens in the mixed treatments below that in the 0.5 g large larvae treatment. There was no significant difference between these treatments (F=4.23; df=2, 14; N.S.) indicating that despite the increased ratio of small to large larvae, no inhibition was detected. Total follicles and final queen weight confirmed this.

Number of pupae. Once a larva has pupated, is it still capable of stimulating fertility? To answer this question, pupae and larvae were tested in the following experiment. Four experimental colonies consisting of 1.5 g workers were set up from each worker source colony, and a queen from a stock colony adopted into each. The treatments applied to these colonies were as

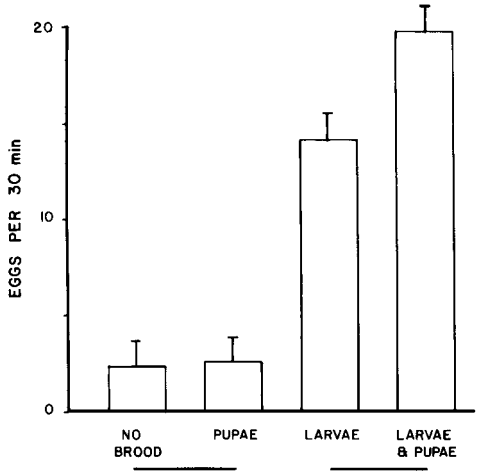

FIG. 9. The effect of pupae, larvae and both on the egg-laying rate of fire ant queens. Mean values for each treatment are indicated on the horizontal scale, and those not statistically different are underscored with a heavy line. Pupae do not stimulate oviposition, either by themselves or in combination with larvae. SEM from pooled ANOVA data indicated by bar.

follows: (1) no brood added; (2) 1.5 g pupae added; (3) 1.5 g larvae added; (4) 1.5 g each of larvae and pupae added together. The experiment was replicated four times with four worker source colonies. Egg-laying rates and other measures were determined 4 days after the treatments were applied.

Treatment had a significant effect on oviposition (F=9.02; df=3, 15; P<0.01) (Fig. 9), but this was due entirely to larvae, not pupae. The egg-laying rate of queens in nests with pupae did not differ significantly from those in broodless nests, nor did the rate differ between queens in nests with larvae and nests with both larvae and pupae (Duncan's Multiple Range Test, P>0.05, N.S.). On the other hand, the presence of larvae significantly stimulated oviposition in comparison with broodless nests or nests with pupae only (P<0.05). It thus follows that the stimulatory effect of larvae disappears as soon as the larvae pupate, or possibly even as soon as pupal apolysis occurs.

Sexual larvae. All experiments to this point were done with worker larvae. During the late spring and early summer, fire ant colonies produce large numbers of sexual larvae, both male and female. Do these larvae have an equally stimulating effect on oviposition as do worker larvae?

Early attempts to set up experiments were blocked by difficulty in maintaining larval numbers at the desired levels. Workers tended to slaughter sexual larvae for unknown reasons. The problem was made less acute by using more workers in the experimental colonies, and by replacing daily those larvae eliminated by the workers, keeping the numbers approximately constant over the 4 day experimental period. For equivalence, worker larvae that disappeared or pupated were similarly replaced with fresh worker larvae. Two experimental colonies each containing 5 g of workers were set up from each worker source colony. A stock queen was adopted into each. The first received 1 g of worker larvae (approximately 1200) and the second 1 g of sexual larvae (approximately fifty-five). Sexual larvae were collected from field

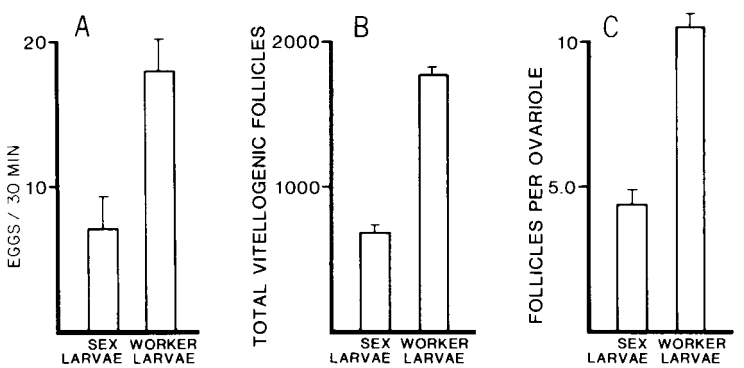

FIG. 10. (A–C) The effect of sexual larvae on the egg-laying rate and ovarian activity of fire ant queens. An equal weight of worker larvae served as a control. Sexual larvae are much less stimulating to oviposition than an equal weight of worker larvae. Error bars=1 SEM from pooled ANOVA data.

colonies and were of unknown sex. The experiment was replicated eight times with eight different worker source colonies.

On a live-weight basis, sexual larvae are much less supportive of oviposition than are worker larvae (F=13.3; df=1, 15; P<0.02) (Fig. 10A). Queens in colonies with sexual larvae laid an average of 7.00 eggs/30 min while those in colonies with worker larvae laid 18.00 eggs/30 min. This is reflected in significantly lower total follicles (F=199; df=1, 15; $P \ll 0.001$) (Fig. 10B), follicles per ovariole (F=74.2; df=1, 15; $P \ll 0.001$) (Fig. 10C) and final queen weight (F=13.0; df=1, 15; P<0.02) of queens in colonies with sexual larvae. Over the 4 day period, twenty to thirty-five (30–60%) of the fifty-five to sixty sexual larvae were replaced, while about 75% of the worker larvae were.

Sexual larvae are only about 5% as stimulatory as worker larvae on a weight basis, but on a per-individual basis they stimulate oviposition to about the same extent (fifty-five worker larvae estimated to result in 8.0 eggs/30 min and 540 follicles; fifty-five sexual larvae result in 7.0 eggs/30 min and 693 follicles). The 1200 worker larvae in the sexual larva experiment gave similar levels of stimulation as 1200 worker larvae in the larval numbers experiment: 18.0 eggs/30 min, 1768 follicles versus 20.3 eggs/30 min, 1440 follicles. This justifies comparison across experiments.

Worker size. Fire ants are a polymorphic species showing large size variation among workers. Some degree of division of labour is associated with worker size (Mirenda & Vinson, 1981; Wilson, 1978). Of special importance here are reports that large workers seem to play a lesser role in brood and queen care (Wilson, 1978). The ability of workers of various sizes to support the stimulatory ability of larvae was therefore tested in experimental colonies composed of three single sizes of workers and a polymorphic control containing 'natural' proportions of each of these three sizes of workers. Workers from large source colonies were separated by size by allowing them to work their way down through a series of geological testing sieves (Nos. 16–35, U.S. Standard Series) (Wood & Tschinkel, 1981). Workers collected on numbers 35, 25 and 18+16 were used as small, medium and large workers, respectively. Workers on numbers 20 and 30 were not used. 1.5 g of workers of each size and of the polymorphic mixture were used for each experimental nest. On the day of the experiment a queen was adopted into each experimental nest, and shortly thereafter 1.5 g of large larvae were added. The experiment was replicated six times with six different worker source colonies, two at a time. Egg-laying was observed on the fourth and fifth days and the queen dissected on the fifth day.

Worker size had a significant effect on two measures of fecundity, total follicles (F=7.08; df=3, 15; P<0.01) (Fig. 11B) and follicles per ovariole (F=5.28; df=3, 15; P<0.05) (Fig.

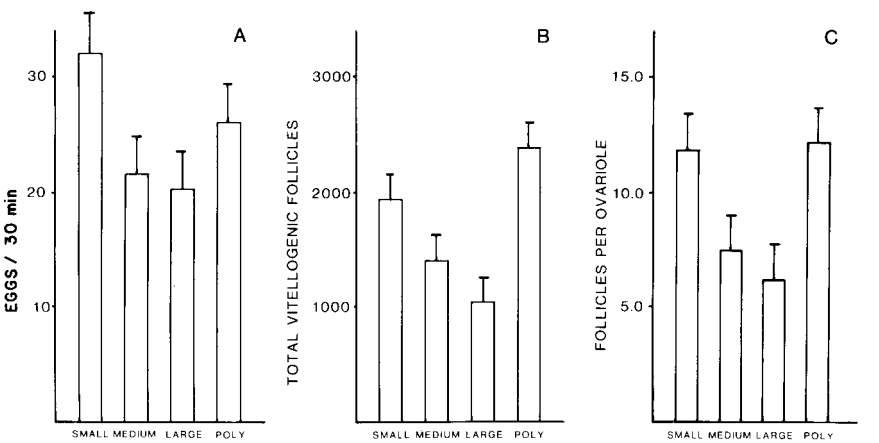

FIG. 11. (A–C) Queen egg-laying rate and ovarian activity in response to varying worker body size. Both total worker weight and total larval weight were kept constant. The control was a standard polymorphic mixture of worker sizes (poly). Workers become less effective at facilitating oviposition as their size increases. Error bars=1 SEM from pooled ANOVA data.

11C), but fell short of significance on eggs/30 min (F=2.50; df=3, 15; N.S.) (Fig. 11A). The pattern of all three measures in relation to worker size is similar, with small and polymorphic nest queens having the highest fecundity, medium worker nests lower and large worker nests, the lowest (Fig. 11A–C). Because the amount of stimulation depends only on the number of larvae and is constant across all treatments, the observed differences must reflect the effectiveness with which the different sized workers transfer the stimulation from the larvae to the queen. This effectiveness declines from small to large workers. The differences cannot be attributed to the decreasing number of larger workers (weight always=1.5 g) because worker number was shown to have no effect in an earlier experiment.

Egg volume and follicle residence time both showed significant relationship to worker size, both being maximum in large-worker nests (F=5.42; df=3, 15; P<0.02; F=5.05; df=3, 15; P<0.05, respectively). The only other measure which was significantly related to worker size was the retinue, but this was an artefact because only a much smaller number of large workers can crowd around the queen, leading to smaller retinues.

Worker age. Fire ants, like other ants, carry out a progression of duties as they age (Mirenda & Vinson, 1981). Young ants generally spend most of their time near the brood and queen, caring for both. As they age, they take on general nest duties and become reserves. Only toward the end of their lives do they leave the nest to become foragers. It therefore seemed possible that the ability of workers to transfer the larval stimulation might vary with worker age, and this was specifically tested in an experiment.

All brood and the queen were removed from four field colonies. These colonies were kept at 30°C for 40 days until the experiment was begun, so that the youngest workers in these stock colonies were at least 40 days old. Each stock colony was divided into three fragments: 1500 workers, 300 workers and the remainder (to be used as a source of workers to replace those that died in the experimental nest). About 1200 pupae were added to the 300-worker nest, and 10 days were allowed for their eclosion ('young' workers). The experiment was then initiated by adopting a queen into each of the eight experimental colonies (four replicates), adding 1.5 g of larvae and observing egg-laying on days 3, 4 and 5. The mean eggs/30 min over the 3 days was used in the ANOVA.

'Young' workers are no more effective in stimulating oviposition than are old workers (37.5 and 27.8 eggs/30 min, respectively) (F=1.46; df=1, 7; N.S.). Deletion of an outlying value brought these means even closer. Workers more than 1 month of age seem to be equally as effective as workers of 1–2 weeks, implying considerable flexibility in division of labour, a flexibility also noted by Calabi (1987). Alternately, perhaps 1 month is still too young for expression of any age-based division of labour.

Shortly after collection of the stock colonies, workers were marked with blue spray paint. At the time of the experiment, only old workers were marked blue, though not all old workers were still so marked. During the observation periods, blue and unmarked workers in the retinue were counted separately. Their proportion in the retinues of 'young-worker' nests was compared to the expected value calculated from the proportion of old workers in the 'young' nests (0.20) and the proportion of blue workers in the old-worker nests (varied from 0.42 to 0.60).

The fraction of blue workers in the retinues of young-worker nests was significantly lower than expected (P<0.02), suggesting that, in the presence of young workers, old workers are less likely to take part in queen care.

Overall correlations. Because most of the dependent variables are functionally related to one another, a large number of possible pairwise correlations among them were significant. During early experiments (worker number, pupa number, worker age) queens were not dissected, so data from these were not used. Table 1 lists a number of pairwise correlations of special interest. All but two are significantly related, explaining 14–89% of the variation. Overall, these correlations confirm what individual experiments have already indicated: that as fecundity (eggs per 30 min) goes up, so does queen weight, follicles per ovariole, total follicles, and time spent feeding. Retinue size shows no significant relation because it is rather variable and not represented by a great range of mean values. Most of the correlations are positive, but the egg volume is negatively related to measures of fecundity such as eggs per 30 min, queen weight,

follicles per ovariole or total follicles. These four correlations are probably not linear because egg volume seems to approach some minimum gradually, but the variation is too high to allow reliable curve-fitting.

Fecundity increase during natural growth. Is the relationship between number of larvae and fecundity quantitatively similar in naturally growing and experimental colonies? For this comparison, newly-mated queens were allowed to establish colonies in the laboratory, and, as they grew, these colonies were monitored in two different ways. In Group A, egg-laying rate and colony census were determined once a week. If a queen died, she was replaced with another of the same age. In this way, twelve colonies were followed over 215 days, although no colony spanned this entire period. Colonies that survived at least 180 days attained sizes of 1500–11,000 workers and up to 6000 feeding larvae. In Group B, egg-laying rate was monitored only once in each colony. The colony was then censused, terminated and the queen dissected. This was carried out on four colonies 3 months of age and nine colonies 6 months of age. These colonies ranged in size from 510 to 9800 workers and up to 1200 feeding larvae.

The data from Group A are mostly repeated measures and are not strictly comparable to the independent data from Fig. 2 and Group B. To make the comparison, data from Group A were analysed as 'slices in time' by dividing the record into 10 day increments such that most colonies appear only once in each 10-day segment. The values within each segment are thus independent. Seven segments were analysed, and the mean and SD of the regression coefficient were used for the comparisons that follow. Ten-day segments earlier than 180 days of age showed very low numbers of larvae and the egg-laying rates were mostly zero. These segments were not included in further analysis.

For all three groups, the number of larvae and the eggs per 30 min were log–log transformed and regressed. Slopes and intercepts were compared using the *t*-test. Over most of the relevant range, a given number of larvae has a significantly lower effect on fecundity in the natural colonies than in the experimental ones (Fig. 12A) (intercept of naturals significantly lower

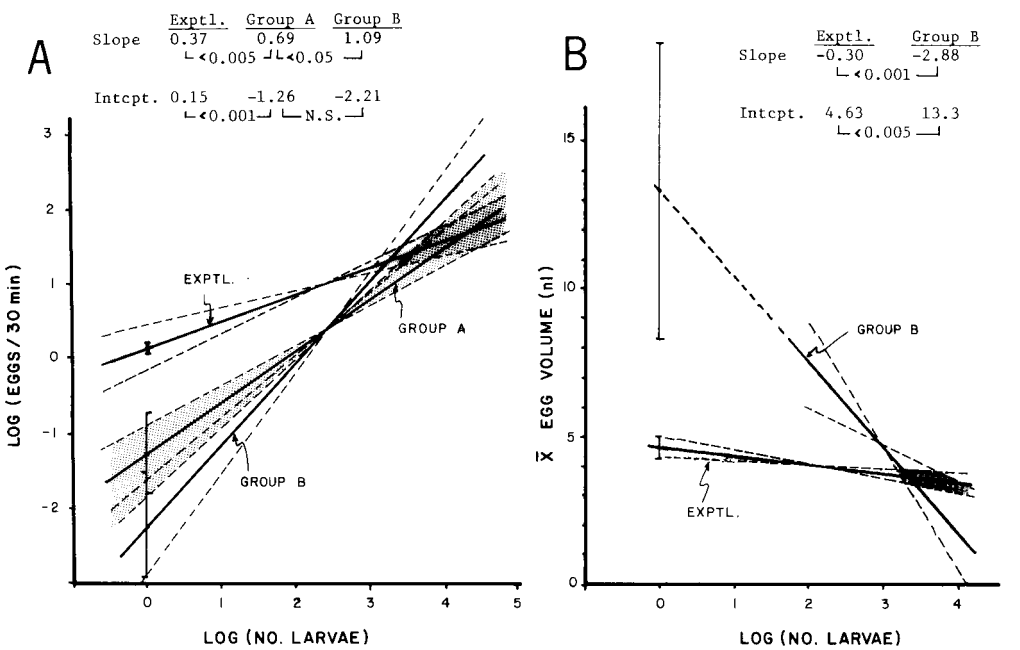

FIG. 12. Comparison of the relationship between (A) egg-laying rate or (B) egg volume (nl) and number of feeding larvae in experimental colonies and two different groups of young, naturally growing colonies. Shaded areas indicate the 95% confidence limits for the slope of each regression. The error bars at log larvae=0 indicate the 95% CI for the intercepts. Coefficients and results of *t*-tests are given in the tables. 'Experimental' data are those from the larval number experiment (Figs. 2 and 3).

than experimental). On the other hand, the factor of increases (i.e. slope) caused by each additional larva is significantly higher in the natural colonies, so that, with more than several thousand larvae, the predicted egg-laying rates are similar. The extrapolation of the Group B regression beyond about 2000 larvae is unlikely to be valid. Field queens laying more than 100 eggs per 30 min have never been found, and it is likely that the slopes of the natural regressions decrease at high larval number (not tested). The significant differences between Groups A and B are difficult to explain. Perhaps they reflect the different methods used or the fact that they were carried out in different years. Despite these problems, we can reliably conclude that the multiplicative effect of larvae on egg-laying rate is greater in natural colonies, and that queens with low numbers of larvae lay fewer eggs in 'natural' laboratory colonies. There are several possible sources for these differences. (1) There could be maturational differences between the experimental queens from mature colonies and the young queens from the natural colonies that cause them to respond quantitatively differently to larvae. (2) The addition of larvae all at once to the experimental nests, rather than their appearance in the course of natural growth, could result in abnormally high stimulation of egg-laying. The subsequent drop in fecundity of experimental queens (Fig. 6) may represent the recovery from this overshoot. (3) The somewhat different independent variables may play a role: in natural colonies, the number of feeding larvae was counted at the same time the queen was observed, but the number of larvae in Fig. 2 is actually the number added on Day 0. When the queen's fecundity was determined on Day 4, the fraction still feeding was related to the number of larvae added (Fig. 5). When only the number still feeding on day 4 was used in the regression, the slope was increased to 0.44 and the intercept decreased to 0.13, but this still does not explain most of the difference between the natural and experimental colonies. (4) Queens in small natural colonies lay much larger eggs than do queens in experimental ones, and their decrease in size with increasing egg-laying rates and numbers of larvae is much more drastic (Fig. 12B and Table 2). Extrapolation of log larvae to zero in natural colonies indicates that broodless queens lay eggs of about 13 nl. This compares reasonably with the eggs of newly-mated, colony-founding queens (17–21 nl; SD=3–6 nl). On the other hand, larva-less queens in experimental colonies lay eggs with a volume of 4.6 nl and show much less decrease in egg volume as egg-laying rate and number of larvae increase (Fig. 12B). Perhaps a maturational process is involved in this difference, or perhaps more time is required for adjustment of egg size. Multiplication of egg volume by eggs per 30 min gives the total volume of egg material laid in each 30 min. While this brings the slopes and intercepts of the regressions versus log number of larvae closer together, it still does not explain most of the differences seen in Fig. 12A. Thus, while it is true that natural queens in small colonies lay fewer, much larger eggs in response to a given number of larvae, egg size does not explain most of the difference in total output of egg material.

In natural colonies, the decline in egg size in relation to number of larvae (Fig. 12B) or eggs per 30 min (Table 2) is unlikely to be linear.

TABLE 2. Pairwise linear regressions of variables from Group B naturally growing colonies. Abbreviations as in Table 1. Only the coefficients with asterisks are significantly different from those of the corresponding regression in Table 1.

y (units)	*x* (units)	*b*	*a*	SD	R^2 (%)	df	*F*-ratio	*P*-value
Total follicles	Eggs/30 min	59	89	387	59	11	18.4	<0.005
Retinue	Eggs/30 min	9.4	2.9	13.3	56	11	16.5	<0.005
Time feeding (min)	Eggs/30 min	0.45	0.63	2.98	55	11	15.6	<0.005
Total follicles	Final queen wt (mg)	−1430	174	247	83	11	61.3	≤0.001
Retinue	Final queen wt (mg)	−32.3	5.1	12.1	64	11	21.9	<0.002
Time feeding (min)	Final queen wt (mg)	−6.64	0.95	3.36	43	11	10.1	<0.02
Egg volume (nl)	Eggs/30 min	7.11*	−0.20*	1.11	43	9	9.0	N.S.
Egg volume (nl)	Total follicles	6.34*	−0.0012	1.42	16	1	2.4	N.S.
Egg volume (nl)	Final queen wt (mg)	8.99*	−0.29	1.33	25	10	4.6	
log Total follicles	log Feeding larvae	0.93	0.67	0.29	42	9	8.14	<0.05

Throughout this study the egg volume was rarely less than 3 nl even at very high egg-laying rates.

Except for the regressions involving egg volume none of the regressions for the natural colonies of Group B (Table 2) was significantly different from those for the pooled experimental colonies (Table 1). Thus, in both types of colonies, total follicles, retinue and time feeding were similarly related to eggs per 30 min or final queen weight. Likewise, the activation of the ovaries in relation to the number of larvae is similar (log total follicles versus log larvae, Table 2 and Fig. 4, *t*-test: N.S.). Interestingly, while no pairwise comparisons were significantly different, almost all intercepts were lower and slopes higher for natural colonies. Perhaps this was a consequence of the higher slope of the egg rate versus number of larvae relationship in Group B.

Founding queens have only about twenty to twenty-five active ovarioles, and only the terminal follicle is vitellogenic in these. By 3 months of age, with about 900 workers and 400 feeding larvae, essentially all ovarioles are active, and there is no further increase in 6-month-old colonies. This agrees with Fig. 4 in which essentially all ovarioles are activated by about 100 larvae. Further increase in fecundity is accomplished by increasing the number of vitellogenic follicles per ovariole and possibly the rate of follicle maturation. All in all, the progress of activation during colony growth seems to follow a sequence qualitatively similar to its activation in relation to larval number (Fig. 4).

There is some evidence that the kind of rapid adjustment to changes in larval number seen under some experimental conditions results in frequent abnormalities such as extrusion of follicles from the calyx or ovarioles into the haemocoel (Fig. 13). If several follicles are extruded in sequence, their size order is reversed from that in the ovariole, because the last and proximal follicle pushed out is always the smallest. Previous activation of ovaries to high levels may be required for rapid readjustment of fecundity. For example, young queens challenged with unaccustomed numbers of larvae frequently extruded follicles into the haemocoel. The exact nature and fate of these hernia-like extrusions awaits histological study.

Larvae as a source of bulk-transferred material. The data suggest that the queen's egg-laying rate may be regulated by a material

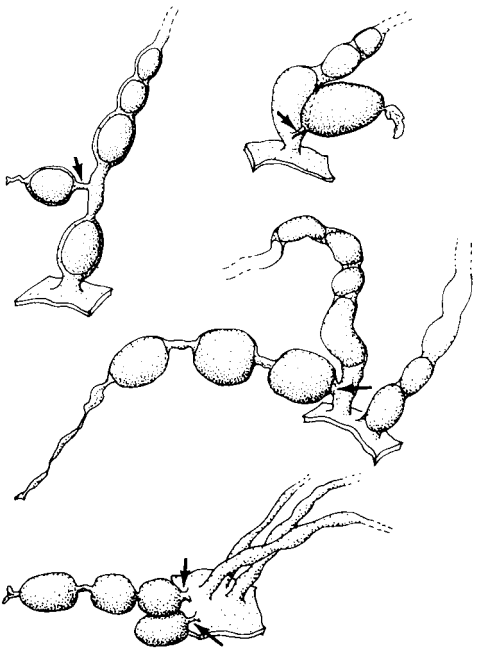

FIG. 13. Extrusion of follicles into the haemocoel through hernias (arrows) in the calyx or ovariole. Extrusion of a series from the ovariole usually results in the largest follicles in the distal position. Only affected portions of the ovary are shown attached to calyx whose lumen lies below. Drawn from specimens preserved in 2.5% glutaraldehyde in insect Ringer's saline.

emanating from the late fourth instar larvae. Existence of such flow was tested using larvae fed vital dye, as described below.

Eight stock colonies were fed a 10% solution of casamino acids (a partially hydrolysed protein fed preferentially to larvae (Howard & Tschinkel, 1981)) which was dyed with 0.5% rhodamine B, a fluorescent vital dye. The next day, the now pink larvae were collected and separated into large and small larvae by sieving. Workers from each (undyed) source colony were divided into four experimental colonies containing 1 g workers each. A queen was adopted into two of these, while two remained queenless. A half gram of large dyed larvae was added to one each of the queenright and queenless nests, and a half gram of small larvae to the other.

Haphazard samples of fifty workers were removed from each experimental colony at intervals after addition of the dyed larvae, anaesthetized with ether, placed between glazed

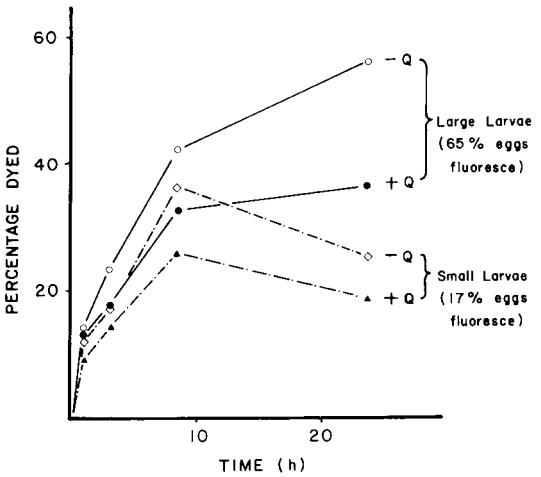

FIG. 14. The percentage of workers (in samples of fifty) containing fluorescence as a function of time after introduction of equal weights of fluorescent-dyed larvae. Dye spreads more rapidly and to larger numbers of workers from large than small larvae. Large larvae also result in a higher fraction of fluorescent eggs after 24 h. Queenlessness raises the level of dyed workers. The SEM for comparisons among Q×L×T cells means is 2.96, for comparisons of L×T cells is 2.10, for comparison of Q×L cell is 1.05 and for T is 2.13

papers crushed with a roller. The papers were then inspected under UV light and the number of ants containing fluorescent material recorded. After 24 h, the eggs were collected from each of the queenright nests and the number fluorescing counted. Four replicates of this experiment were carried out, two at a time.

The results (Fig. 14) show that dyed material moved rapidly from the larvae to the crops of workers, to the queen and into the eggs. This flow was significantly more rapid for colonies given large larvae than small (effect of larval size: $F=20.5$; df=1, 9; $P<0.02$; larval size×time interaction: $F=11.5$; df=3, 9; $P<0.002$; effect of time: $F=59.5$; df=3, 9; $P\ll0.001$), and for queenless colonies as opposed to queenright ones ($F=12.0$; df=1, 9; $P<0.05$). 9–13% of worker crops contained fluorescence within 45 min. Fluorescence appeared more rapidly and was more widespread in colonies with large larvae implying that the flow per unit weight is higher from these than from small larvae. Dyed crop frequency builds up faster and to higher levels in queenless nests, implying that queens act as a sink for the dyed larval material. This is supported by the fact that 65% of the eggs laid by queens in colonies with large larvae are fluorescent after 24 h, while only 17% fluoresce in colonies with small larvae.

Assuming that the larvae of different sizes do not segregate the dye differently, it is clear that weight for weight, large larvae produce much more of a material which flows from them via workers to the queen and into the eggs. Whether this material is or contains the agent controlling queen egg-laying is presently unknown.

Discussion

The social control of queen fecundity in *S. invicta* is unlike that in any other social hymenopteran species investigated to date. While queen fecundity in *Plagiolepis pygmaea* is dependent on workers, but not larvae (Passera, 1972), that of *Formica fusca* is suppressed by larvae (Bier, 1954; Mamsch, 1967), that of *Myrmica* is not strongly dependent on either (Brian, 1957a, b, 1969) and that of honeybees and stingless bees depends upon empty brood cells (Free, 1977; Sommeijer, 1985), queen fecundity in *S. invicta* is regulated through a highly specialized dependence on larvae. The queen's egg-laying rate is linked by a positive feedback to the number of fourth instar larvae, such that fecundity is driven to ever higher levels as eggs develop into fourth instar larvae. This feed-back must contribute substantially to the observed exponential growth (Tschinkel & Howard, 1983) of young fire ant colonies. The duration of the stages is such that when the hatchlings from eggs need care, the fourth instar larvae which stimulated the production of those eggs are available as newly eclosed workers. Brian (1957b) noted a similar coincidence in the duration of stages in *Myrmica*.

Fire ant queen fecundity further differs from all the above examples (except perhaps the honeybee) in being much higher. For example, a queen of *M. rubra* lays about 628 eggs per year (Brian & Hibble, 1963). At her peak, a fire ant queen can lay this number of eggs in 3–4 h. Such high oviposition rates are necessary to achieve large colony size and rapid colony growth in this species (Tschinkel, 1986). Colony size in *S. invicta* is far in excess of that of 'ordinary' ants and undoubtedly represents a specialization. The mode of fecundity control may in turn

represent a specialization associated with large colony size. The important comparisons are thus other species with large colonies, rather than species with average colony size. Because large colony size has evolved independently in a number of ant taxa, the mode of achieving and controlling high queen fecundity rates may differ among these. At this time, data are lacking.

In monogynous species, the fecundity of social insect queens is related to maximum colony size and varies enormously among species. Higher potential fecundity evolves through an increase in the number of ovarioles (Wilson, 1971). Among the ants, queens have as few as six, the basic aculeate number, as many as 200 in *S.invicta*, 300 in *Atta texana* (Tschinkel, 1987), 2400 in *Eciton burchelli* (Hagan, 1954) or 14,000 in *Dorylus* (*Anomma*) *nigricans* (Schneirla, 1957). Queens with few ovarioles are probably similar to non-social aculeates in fecundity, while *Eciton burchelli* queens can lay 58,000 more eggs per batch (Hagan, 1954) and *D.nigricans* up to 1 million (Schneirla, 1957). Fecundity is also increased by laying smaller eggs, as in *Myrmica* (Weir, 1958; Brian & Hibble, 1963) and *S.invicta* (Fletcher & Blum, 1983), and by more rapid maturation of follicles. The latter has been suggested as the cause of the former, but in my experiments follicle maturation time (total follicles/eggs per 30 min) rarely showed a significant relationship to the egg-laying rate. A direct experimental test is needed. Fecundity is also increased by an increase in the number of maturing follicles per ovariole, and this mode is probably regulative and universal.

A fecund fire ant queen is capable of an impressive daily output of eggs. A queen laying at the average rate associated with 10,000 larvae in Fig. 2 lays about 7 mg of eggs per day. Total weight of such a queen is typically about 24 mg. A number of queens fresh from field colonies were observed to lay at up to twice this rate, and to weigh 25–29 mg. 50–70% of this weight was ovaries, for queens with regressed ovaries (i.e. in broodless nests) generally stabilize at about 9 mg. These figures underscore the view that the queen is an egg-laying machine, the evolutionary victim of an extreme division of reproductive labour.

In the present interpretation fire ant workers are primarily the conduits between the fourth instar larvae and the queen. To some degree they can also act as reservoirs for the larval material for limited periods (unpublished data). Unlike workers in some other ant species they cannot stimulate fecundity by themselves. Their effectiveness as conduits is related to their body size: the larger the fire ant worker, the less effective, at least in monomorphic colonies (Fig. 11). This parallels their ability in brood rearing (Porter & Tschinkel, 1985b), and is an aspect of division of labour by size among workers (Wilson, 1978). Worker age, on the other hand, had no effect on the ability to transfer the stimulation from larva to queen, in spite of the fact that most species of ants work as nurses when young and progress to general nest duties and foraging as they age. The absence of an effect could have resulted from insufficient age differences, or behavioural flexibility of workers in uniform-aged colonies (Calabi, 1987).

The broad outlines of fecundity-increase during field colony growth and maturity can be extrapolated from my experiments, but a good deal of quantitative uncertainty remains, particularly concerning the per-larva power of stimulation, and therefore the rate of fecundity change during growth of the larval population (Fig. 12). While it is clear that queens are capable of changing fecundity within 24 h or less in response to changes in larval populations, such changes are probably gradual in field colonies, occurring most obviously during colony growth through gradual activation of the queen's reproductive system. In very young queens all ovarioles are rapidly activated by the increasing larval population, after which all increase in fecundity is the result of higher output from each ovariole. At the same time, egg size declines from the relatively huge eggs laid by founding queens to about one-fifth of this size, increasing fecundity through more parsimonious use of egg material.

Fecundity also fluctuates strongly with the seasons, in part as a result of the interaction of temperature with larval stimulation and other factors. One factor which may contribute is production of sexual brood during the spring. On a weight basis, the fecundity-stimulating power of sexual larvae is only about 5% that of worker larvae, though on an individual basis they are about the same. During the spring, worker production declines and colony biomass may consist of 10% sexual brood (Markin & Dillier, 1971). At this time, the queen's fecundity probably must be reduced because adult sexuals do not

participate in brood care, and the worker force is occupied with rearing sexuals. The lower stimulation provided by sexual larvae automatically adjusts the queen's egg laying to the appropriate lower levels.

As social insect colonies approach their species-typical maximum size, density-dependent feedback or emigration of sexuals or both slows growth rate until it equals zero or declines. In fire ants, this slowing of growth rate may be partially the result of the declining efficiency of larvae in stimulating egg-laying by queens, the declining efficiency of brood rearing by workers (Porter & Tschinkel, 1985b), or the physical limits of the queen. Several species of social insects show declining reproductive efficiency with colony size (Brian, 1983; Michener, 1964), and this may be a universal trait in insect societies. Brian (1956) suggested that in *Myrmica*, worker inefficiency during brood rearing increases with colony size as the net outcome of the underdispersion of larvae and the test-servicing system of larval care. The cause of the decline of reproductive efficiency in fire ants is not known. In any case, continued growth requires that births exceed deaths, but while deaths are approximately a fixed proportion of the colony population, births are a declining proportion as a result of declining reproductive efficiency. When birth and death rates are equal, the colony is at its maximum size. Maximum queen fecundity is clearly a central parameter determining maximum colony size in monogynous species.

By what mechanism do fourth instar larvae occupy this key position in colony reproduction? Brian & Brian (1948) and Schneirla (1957) hypothesized that larvae have feeding priority over queens, causing oviposition to increase when larvae stop feeding and enter metamorphosis. Oviposition rate should therefore be related to the worker/larva ratio. Evidence for *S.invicta* fails to support this mechanism because oviposition rate is independent of worker/larva ratio across the entire tested range of 16-fold, and depends only on the number of larvae. A more appealing hypothesis is as follows. The fourth instar larvae are the only colony members that are able to ingest and digest the solid prey protein collected by the workers (Petralia & Vinson, 1978; Petralia *et al.*, 1980). All other colony members (workers, queen and first to third instar larvae) feed only on filtered pre-digested and concentrated foods or foraged liquids. In fact, workers are unable to ingest particles larger than 1 μm (Glancey *et al.*, 1981). Workers have little need for protein, so protein is directed primarily toward the larvae and queen (Howard & Tschinkel, 1981; Sorensen & Vinson, 1981) who need large amounts for growth and egg-production, respectively. The fourth instar larvae are thus a key 'metabolic caste' making liquefied protein, including enzymes which workers may lack, available to the queen and younger larvae. The finding of high concentrations of amino acids and protein in the labial gland secretions of fourth instar larvae (Sorensen *et al.*, 1983b), and the movement of dye from such larvae to worker crops and into the eggs (Fig. 14), are in accord with such a mechanism. Workers have been reported to collect oral material from larvae (Sorensen *et al.*, 1983a; O'Neal & Markin, 1973). It is also possible that anal materials are important.

The material flowing from the larvae to the eggs may be digestive enzymes, simple nutrients (e.g. amino acids), special pheromones or all three. Possibly, flow of bulk material is merely rate-limiting, with a set-point determined separately by a pheromone or other factor. More speculatively, the larvae might synthesize vitellogenin or its precursors which the queen then merely packages into the eggs. This hypothesis has been suggested, but not supported, for honeybees, whose workers contain vitellogenin in their haemolymph (Rutz & Lüscher, 1974). Honeybee queens seem able to synthesize enough vitellogenin to lay their own weight in eggs daily (Engels, 1972).

In view of the role of larvae in *S.invicta*, a report of special interest is that of Masuko (1986) on *Amblyopone silvestrii*. In this and several other primitive species, queens subsist exclusively by feeding non-destructively on haemolymph gained by biting their larvae. Could this be an evolutionary antecedent of the system in *S.invicta*? Identification of the fecundity-stimulating material in *S.invicta* should illuminate evolutionary questions such as this.

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