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**Abstract.** A comparison of several physiological parameters of queens of *Iridomyrmex humilis* in experimental monogynous and polygynous colonies showed that queens in monogynous colonies became heavier, had more developed ovaries and laid about twice as many eggs. Workers in monogynous colonies were more attracted to queens, which therefore probably received more food. This may partially explain the higher weight and fecundity of queens in monogynous colonies of *Iridomyrmex humilis* and possibly other ant species. In polygynous colonies, queens differed greatly in their fecundity. These differences did not appear to be the result of a dominance hierarchy. These results are discussed from an evolutionary point of view. Two hypotheses of mutualism and colony level selection are proposed as an alternative to kin selection which is unlikely to be the exclusive selective influence in the evolution of polygyny either in *I. humilis* or in most other ant species.

Following the conventional models of evolution (Fisher 1930; Wright 1931; Haldane 1932), selection acts in favour of the genomes of individuals able to produce many surviving offspring. To increase this production, individuals compete among themselves. In social insects, cases of competition are well documented (Barron-Urban 1968a; Wilson 1971; Keller & Chérix 1985).

In the case of polygyny (the presence of more than one egg-laying queen in the same colony, Wilson 1974b), several authors have shown that queen fecundity is inversely proportional to their number in the colony (e.g. Michener 1964; Enright 1969; Passera 1969; Roisin & Pastor 1985). To explain this decrease in queen fecundity, Fletcher et al. (1980) proposed two hypotheses. In *Solenopsis invicta*, the lower ratio of workers to queens found in polygynous nests compared with monogynous nests may create resource limitation for the queens, or alternatively, in nests, queens may possess a pheromonal mechanism which mutually inhibits their fecundity.

The lower fecundity of queens in polygynous colonies raises the question whether the decrease in fecundity affects all queens equally or whether it leads to the formation of a dominance hierarchy among queens. In this paper, the Argentine ant, *Iridomyrmex humilis* (Mayr), a typically polygynous species (Newell 1909; Markin 1970a), was chosen to investigate the causes of lower fecundity of queens in polygynous colonies and was used as a test of the existence of a dominance hierarchy among queens.

**MATERIALS AND METHODS**

Seven stock colonies of *I. humilis* were collected in March 1983 in the south of France on the Mediterranean coast between Nice and St Raphael. The colonies were maintained in the laboratory in artificial nests (Passera et al., in press) at 27 ± 2°C and at a relative humidity of 55 ± 5%. They were fed ad libitum on a diet of meat, eggs, sugar and vitamins. Mealworms were given regularly. Each of the seven stock colonies was split into one polygynous colony, containing between five and eight queens, and a control group containing between five and eight monogynous colonies (the number of monogynous colonies in each control group was equal to the number of queens in the corresponding polygynous colony). Altogether, 42 queens were distributed in seven polygynous colonies and 42 queens in the seven control groups. Each queen was marked with Tech pen ink (Marktex) and given 0-9
cm² of workers and brood (about 600 workers). At 10, 20, 30, 50, 80, 120 and 160 days after the beginning of the experiment, each queen was weighed and subjected to an ovispersion test. In this test, each queen was isolated during 14 h in an experimental nest and the number of eggs laid during this period were counted under a dissecting microscope.

When a queen in a polygyrous colony died, a queen of the corresponding group of monogynous colonies was randomly chosen and eliminated. The same procedure was used when a queen in a monogynous colony died. Consequently, the number of queens remained equal in both kinds of colonies.

On day 160, the 28 remaining queens in monogynous colonies and the 28 queens in polygyrous colonies were tested for their attractiveness to workers in a manner similar to that described in Cogliore & Cammaerts (1981). Each queen was isolated in a plastic box (8.0 cm² diameter) with 50 workers randomly chosen from her colony. The plastic box was closed with a lid to avoid disturbance and the inner side was coated with Fluon to prevent workers and queens from escaping. Ants were allowed 15 min to acclimatize after which time the number of workers in contact with the queen was counted once every 5 min for 1 h (12 values). The same procedure was repeated for each queen once in the morning and once in the afternoon, with different groups of workers. The attractiveness of each queen was quantified by calculating the mean of these 24 values.

Finally, each queen was dissected. Ovarian development was estimated from the number of mature oocytes (more than 0.25 mm long).

**RESULTS**

**Queen Weight and Fecundity**

Queen weight increased during the first 80 days in both monogynous and polygyrous colonies (Fig. 1). But the increase in monogynous colonies was more rapid, and queens from monogynous and polygyrous colonies differed significantly in weight from day 30 onwards.

Queen fecundity increased up to day 80 in both monogynous and polygyrous colonies (Fig. 2). The increase in queen fecundity was again greater in monogynous colonies; the ratio of the mean rate of egg laying by queens in monogynous colonies to

![Figure 1. Mean queen weight (± SD) of queens in polygyrous colonies (open circles) and monogynous colonies (closed circles). *P < 0.05; **P < 0.01 indicate significant differences between monogynous and polygyrous colonies (t-test). The number of queens in monogynous and polygyrous colonies was equal: N = 42 at day 10, 20, 30, 50; 38 at day 80; 33 at day 120; and 28 at day 160.

![Figure 2. Mean number of eggs (± SD) laid by queens in polygyrous colonies (open circles) and monogynous colonies (closed circles). The number of queens is as in Fig 1; *P < 0.05; **P < 0.01; ***P < 0.001.

![Figure 3. Distribution of number of mature oocytes per queen for 28 queens reared 160 days in polygyrous colonies and 28 queens in monogynous colonies. Queens in polygyrous colonies: \( \bar{X} \pm SD = 7.0 \pm 4.6 \). Queens in monogynous colonies: \( \bar{X} \pm SD = 15.2 \pm 7.5 \). The difference between both means is significant (t = 8.85, df = 56, P < 0.001).

The mean rate of egg laying by queens in polygyrous colonies was 5.1 ± 2.1 eggs per day, while in monogynous colonies it was 9.4 ± 4.6 eggs per day. This difference was significant (t = 3.5, df = 79, P < 0.001).

**Attractiveness of Queens**

The tests for the 160 showed that workers were more attracted to polygyrous queens than to monogynous queens: \( \bar{X} \pm SD = 7.0 \pm 4.6 \) in polygyrous colonies (5.5 ± 1.2; Fig. 1).

**Queen Indicators**

To test the hypothesis that workers use cues from the queen to determine which nest to join, five polygyrous colonies and five monogynous colonies were set up. Queens from control groups were removed from their colonies. If...
Table 1. Volumes of workers plus brood per queen (X ± SD) after 160 days of the experiment

<table>
<thead>
<tr>
<th>Colony</th>
<th>Number of queens</th>
<th>Volume of workers plus brood per queen (cm³)</th>
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<tbody>
<tr>
<td>P₁</td>
<td>5</td>
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<tr>
<td>P₂</td>
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<td>P₅</td>
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<td>P₆</td>
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<td>P₇</td>
<td>8</td>
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<td>Mean</td>
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<th>Colony</th>
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<td>Mean</td>
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<td>1-5 ± 0-4</td>
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* * * P < 0.001

Keller: Evolutionary implications of polygyny

The mean rate of egg laying by queens in polygynous colonies was 160% on day 50, 190% on day 80, 190% on day 120 and 191% on day 160. A t-test showed a significant difference in individual queen fecundity between monogynous and polygynous colonies as early as day 50.

At the end of the experiment, the dissection of queens showed that the mean number of oocytes per queen was significantly greater in monogynous colonies than in polygynous colonies (Fig. 3). This difference in ovarian development of queens was associated with a significantly higher production of workers and brood in monogynous colonies. The volume of workers plus brood per queen increased by 11% between day 0 and day 160 in polygynous colonies, whereas this increase reached 67% in monogynous colonies (Table 1).

Attractiveness of Queens in Monogynous and Polygynous Colonies

The tests for attractiveness performed on day 160 showed that workers were significantly more attracted to queens in monogynous colonies (X ± SD = 7-0 ± 1-6) than in polygynous colonies (5-5 ± 1-2; Fig. 4).

Queen Interactions

To test the interactions among queens and to determine whether a dominance hierarchy exists, five polygynous colonies, each containing five queens on day 120, were compared with five control groups each consisting of five monogynous colonies. If there was a dominance hierarchy among queens in polygynous colonies, there should be larger differences in fecundity between queens in polygynous colonies than within the control groups in which queens were not together.

To study the differences in fecundity among queens, the five queens in each polygynous colony were ranked according to the total number of eggs laid during the six oviposition tests (see Materials and Methods; 1 = queen with the highest egg-laying rate; 5 = queen with the lowest egg-laying rate). The number of eggs was expressed in relation to this ranking system. The same procedure was applied to the five queens in each of the five control groups. Because the same data were used to generate both axes in this analysis, there was of course a negative
Working on the same species, Bartels (1983) failed to show a difference in brood production between monogynous and polygynous colonies. However, a close examination of his results shows that egg production per queen in monogynous colonies was also about twice that in polygynous colonies but, due to the small sample (N = 5) studied, he was not able to show a significant difference.

Figure 5. Mean number of eggs laid by queens as a function of the ranking system of fecondity. Open circles: queens in polygynous colonies; closed circles: queens in control groups of monogynous colonies.

Queen Hierarchy in Polygynous Colonies

In mature polygynous colonies, the existence of a hierarchy among queens resulting from reproductive competition can be tested by comparing their reproductive success, i.e., the number of males, queens and fertile workers they produce. In the case of I. humilis, I have not considered workers, because they never lay reproductive eggs (Markin 1970b; Benois 1973; Bartels 1983). In this study, the fecondity of queens was used as the criterion of their reproductive success, because, for the following reasons, the eggs laid by all queens have the same probability of developing into sexuals. First, all queens lay male-eggs in a similar ratio (Passera et al., in press; Passera & Keller, unpublished data) and second, the development of female-eggs produced by queens into workers depends only on extrinsic factors such as queen inhibition and food status of the colony (Passera & Keller, unpublished data). Consequently, the female-eggs laid by each queen in a colony are equally likely to develop into new queens.

The comparison of the fecondity of queens shows that in polygynous colonies of I. humilis, there is no evidence of a dominance hierarchy between queens. Instead, the difference in fecondity of queens, in polygynous as well as in control groups of monogynous colonies, results from intrinsic physiological differences between the queens. This indicates that the demonstration of important physiological differences between individuals in a colony does not provide conclusive evidence of a dominance hierarchy. To demonstrate the existence of a dominance hierarchy, it is necessary to show that behavioural or physiological differences result from interactions among individuals. This can be done by showing, for example, that these differences disappear when the individuals are separated or that these differences do not exist when comparing individuals that are not interacting. In Leptothorax curvispinosus (Wilson 1974a, b), queens eat the eggs laid by other queens (Evesham 1984) and in Polybia (Wheeler 1984) there is evidence that queens inhibit the females of other queens. The differences between queens given above may possibly explain these differences, as in our experimental conditions. The conclusive evidence for the viability hierarchies resulting from the dominance between queens in mature colonies.

Queen Fecondity

In the experimental group, most of the queens was in polygynous colonies. There is a hierarchy among queens to queens creates a resource competition which inhibits egg laying in the nest. In Leptothorax griseus, Myrmecina grahami (Wilson 1976; Formica ex subnitidus (Wilson 1976), Solenopsis invicta (Wilson 1976), Formica ex hirta and Leptothorax provanchi (Wilson 1980), several queens coexisting in one of them inhibits the fertility. This functional monogynous hierarchy inhibiting competition between queens is common. The fecondity of all queens is reduced equally and there is no hierarchy with one or several queens inhibiting the fertility of the others. The lower fecondity of queens seems difficult to explain. The results of the test of the effect of the presence of workers suggest a new experiment: weight and fecondity of queens in colonies of I. humilis. Queens from the workers (Markin...
species. Bartels (1983) found no difference in brood production between monogynous and polygynous colonies. Although his results show that the queen in monogynous colonies has twice that in polygynous colonies, in the small sample (N = 5) he did not use enough to show a significant difference.

Polygynous Colonies

In this context, the existence of a polygynous colony, resulting from reproduction by comparing their females, the number of males, and the workers they produce. In the case of monogyny, not considered workers, which do not reproduce (Markin et al. 1983). In this study, the percentage of females is used as the criterion of reproduction, because, for the following reason, all by all queens have the potential to develop into sexuals. First, there is a similar reason (Passera & Keller, unpublished data) the development of female-eggs produced by queens or workers depends on the presence or absence of workers such as queen inhibition or worker stimulation. Consequently, the female-eggs produced by colonies are equally likely to develop into sexuals.

The fecundity of queens in polygynous colonies is a measure of the dominance hierarchy and the difference in fecundity in monogynous as well as in control polygynous colonies, results from the difference between the effects that the demonstration of dominance differences between individuals does not provide conclusive evidence of dominance hierarchy. To demonstrate that behavioural or physiological effect from interactions among colonies can be done by showing, for differences disappear when comparing individuals that are in a Leptoetharax curvispinosus (Wilson 1974a, b). queens differ in their tendency to eat eggs laid by other queens. In Myrmica rubra (Evesham 1984) and in Proceripteris scabricauda (Wheeler 1984) there are behavioural differences between queens. However, the behavioural differences between queens in the three examples given above may possibly be the result of individual differences, as in our experiments, rather than the consequence of a dominance hierarchy. In Plagiolepis pygmaea, queens reared in experimental colonies displayed no significant difference in their fecundity (Mercier et al. 1985). Hence, there is no conclusive evidence for the existence of reproductive hierarchies resulting from a dominance between queens in mature polygynous colonies of ants.

Queen Fecundity

In the experimental groups, the ratio of workers to queens is the same in monogynous and polygynous colonies. Therefore the hypothesis that in polygynous colonies the lower ratio of workers to queens creates a resource limitation for the queens (Fletcher et al. 1980), is not adequate to explain the differences in queen fecundity under my experimental conditions.

The second hypothesis (Fletcher et al. 1980) was that queens may possess a pheromonal mechanism which inhibits egg laying by other queens in the nest. In Leptoetharax greleri (Buschinger 1968), Myrmecina graminicola (Baroni-Urbani 1968b), Formica onis (Schefflin & Winter 1967), Solenopsis invicta (Tschookel & Howard 1978), Formica hirticornis (Buschinger 1979) and Leptoetharax provincheri (Buschinger et al. 1980), several queens coexist in the same nest, but one of them inhibits the fecundity of the others. This functional monogyny results from reproductive competition between queens. In I. humilis, the fecundity of all queens in polygynous colonies is reduced equally and there is no dominance hierarchy with one or several queens preferentially inhibiting the fecundity of the others. Therefore, the lower fecundity of queens in polygynous colonies seems difficult to explain in terms of reproductive competition.

The results of the test of queen attractiveness to workers suggest a new explanation for the lower weight and fecundity of queens in polygynous colonies: in I. humilis, queens receive nearly all food from the workers (Markin 1970b). The data in Fig. 3 show that queens in polygynous colonies are less attractive to workers. Thus, these queens have fewer contacts with workers and consequently probably receive less food. This hypothesis, that there is a difference in the amount of food that queens receive, is supported by the fact that the increase in weight of queens was far more rapid in monogynous than in polygynous colonies (Fig. 1). Consequently, the differences in the amount of food that queens receive in monogynous and polygynous colonies may at least partially explain their differences in fecundity.

Polygyny in an Evolutionary Context

In ants monogyny is probably the primitive condition and polygyny derived (Brian 1983; Fletcher & Ross 1985). Following the theory of Hamilton (1964a), polygyny could be explained if queens are related (Hamilton 1964b; Wilson 1966, 1971; Hölldobler & Wilson 1977). In I. humilis, the colony structure is polydomous (i.e. the population is divided into many nests, but they are interconnected). Furthermore, nest sites often shift, split or fuse several times in the year in response to desiccation, flooding or human disturbance (Newell & Barber 1983). Queens are also very nomadic and frequently change nests (Newell & Barber 1983). Therefore, it seems unlikely that queens from the same nest are closely related. In some other ant species, there is a significant relatedness among the nestmate queens, but this relatedness is generally low (Craig & Crozier 1979; Pamilo & Varvio-Aho 1979; Pamilo 1981, 1982; Ward 1983; Crozier et al. 1984; Pamilo & Rosengren 1984), whereas in some other species, no significant relatedness can be detected in most nests (Craig & Crozier 1979; Pamilo 1981, 1982; Pearson 1982, 1983; Ross & Fletcher 1985).

Thus, in I. humilis and in other species where relatedness is probably low, the maintenance of polygyny seems difficult to explain exclusively in terms of kin selection. Another possibility to consider is that polygyny is not the result of altruism. In I. humilis as in many other polygynous species, polygyny is linked to important behavioural and ecological modifications, namely, loss of nuptial flight of the queens, mating within the nest, colony founding by budding, loss of territorial boundaries and aggression among colonies (Hölldobler & Wilson 1977). In I. humilis, these modifications seem very adaptive for the opportunistic
nesting strategy used: frequent moving, splitting and fusion of colonies. Furthermore, in this and probably other ant species, colony founding by budding necessitates a much lower energy investment, which is adaptive in poor habitats (Keller & Passera, in press). Polynyga also seems well-suited for particular patches of habitat (Hölldobler & Wilson 1977; Pamilo & Rosengren 1984). Thus, the social organization adopted by polynygous species may present some selective advantages. This raises the question whether selection acts at the individual level (i.e. queens), or at a higher level (i.e. colony). Under selection at the individual level, the evolution and maintenance of polynyga assumes that for queens the association with other queens outweighs the costs (i.e. loss of personal fecundity). This mutualism has been invoked to explain the temporary association of queens during colony founding in social insects (e.g. Lin & Michener 1972; Bartz & Hölldobler 1982). However, in the majority of cases, either queens begin to fight when the first workers are ready to pupate or emerge, or the first workers to emerge eliminate queens until only one remains (Bartz & Hölldobler 1982). This strong aggression indicates that mutualism does not prevent reproductive competition from operating among queens during the maturation of the colony. In the case of permanent polygyny, it is still difficult to assess whether the benefits for queens of associating with other queens may outweigh the cost in loss of personal reproduction in mature colonies. The alternative explanation invokes selection at the colony level. This mode of selection has been hypothesized by Sturtevant (1938) then discussed by Lewontin (1970), Oster & Wilson (1978), Crozier (1979), Starr (1979) and West-Eberhard (1981) as a means of explaining the evolution of eusociality. Under this mode of selection there may be a selective pressure against competition among queens in polynygous colonies. In which queens compete would probably be at a disadvantage in comparison with other colonies. Although it is difficult to assess the relative importance of these alternative hypotheses, in colonies in which the degree of relatedness among queens is low, selection at the colony level is probably an important selective influence acting against competition among nestmate queens. Selection at the colony level may therefore act in concert with mutualism for the evolution of polynyga when this mode of social organization is best suited to a habitat.

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