

Gyne development in the Argentine ant *Iridomyrmex humilis*: role of overwintering and queen control

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Abstract. A possible stimulatory effect of overwintering on gyne development in *Iridomyrmex humilis* (Mayr) was investigated. Although gyne-potent larvae are present in the nest throughout the year, small queenless colony fragments composed of freshly overwintered ants (collected in late winter or early spring) produced 3–8 times more gynes than comparable fragments made up of non-freshly overwintered ants (collected at other times of the year). Apparently, this stimulatory effect of overwintering acts on both the developmental potential of larvae and the tendency of workers to rear sexually competent larvae as gynes; queenless colony fragments in which either the brood or workers were freshly overwintered produced more gynes than fragments composed of non-freshly overwintered workers or brood but fewer than fragments containing both brood and workers freshly overwintered. This increased sexualization potential of larvae due to overwintering is enough to overcome weak pheromonal inhibition of queens associated with low queen number; a single queen in a large freshly overwintered colony fragment is insufficient to inhibit gyne development, whereas ten queens are totally inhibitory. In non-freshly overwintered colony fragments one queen is completely inhibitory. Variability in egg developmental potential according to queen age does not appear to play a major role in the seasonal production of gynes, because at least some eggs of very young queens (less than 3 weeks old) are capable of gyne development. In the field this stimulatory effect of overwintering is superimposed on a seasonal fluctuation in the combined strength of pheromonal queen control. In southern France, gynes are produced only in spring where they arise primarily from overwintered larvae just after a sharp drop in queen number, and presumably the total level of inhibitory queen pheromone, due to the massive execution of queens by workers.

Key words. Gyne production, caste determination, overwintering, queen control, ants.

Introduction

A characteristic feature of social insects is the division of labour into reproductive and non-reproductive (worker) castes. The process whereby the development of individual colony members is channeled into one caste or another is complex and subject to a number of external controls, including both social and environmental factors (reviewed in Wilson, 1971; Brian, 1979, 1980; Passera, 1984; Fletcher & Ross, 1985; Wheeler, 1986; Hölldobler & Wilson, 1990;

Passera & Suzzoni, 1991). Among ants, several factors have been implicated in caste determination, the most widespread of which appear to be an inhibitory queen influence and a stimulatory effect of overwintering. For example, in the myrmicines *Myrmica rubra* and *Leptothorax nylanderii* and the formicine *Plagiolepis pygmaea* only female larvae that have overwintered in the nest are capable of development as new queens (gynes), and this development is inhibited by the presence of egg-laying queens, probably by means of a pheromone (Brian, 1979, 1980; Plateaux, 1970, 1971; Passera, 1969, 1980a). In the myrmicine *Pheidole pallidula* and the formicine *Formica polyctena*, neither of which overwinters brood, gynes are

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reared from the first batch of eggs laid in spring; these eggs differ in size and chemical composition from eggs laid later in the season and are more gyne-potent (Bier, 1953, 1954; Gösswald & Bier, 1953, 1954a, b; Passera, 1980b; Suzzoni *et al.*, 1981). Circumstantial evidence (Gösswald & Bier, 1954b; Passera, 1980b) suggests that queens of both these species may exert an inhibitory influence on gyne development.

Caste determination in the Argentine ant, *Iridomyrmex humilis* (Mayr) (Dolichoderinae), has recently been shown (Vargo & Passera, 1991) to be inhibited by queen-produced pheromones. Gyne production in this polygyne species (many egg-laying queens per nest; Newell & Barber, 1913; Markin, 1970; Keller *et al.*, 1989a) occurs only in spring, after a massive execution of queens by workers (Markin, 1970; Benois, 1973; Bartels, 1983; Keller *et al.*, 1989a). This regicidal act presumably lowers the level of queen pheromone permitting some female larvae to develop as gynes. In addition to this pheromonal queen control, season appears to affect gyne production in *I. humilis*, at least in southern France where gynes develop primarily from overwintered larvae (Benois, 1973; unpublished data). Here we report on investigations of the influence of overwintering on gyne production in this species and of the possible effect of season and queen age on the strength of queen control.

Materials and Methods

Source and maintenance of ants. The ants used in these studies originated from Port Leucate on the Mediterranean coast of France and were collected and removed from the soil as described by Passera *et al.* (1988a) and Keller *et al.* (1989a). Colonies were housed in artificial nests (Passera *et al.*, 1988a) and maintained in the laboratory under summer conditions ($28 \pm 2^\circ\text{C}$, LD 16:8). Food, in the form of an artificial diet (Keller *et al.*, 1989b), and sugar water were provided *ad libitum*.

Colonies were classified as either freshly overwintered or non-freshly overwintered, depending on the time of year collected and the length of time maintained under summer conditions in the laboratory before experimental use. Colonies fresh from the field (used for experiments 7–15 days following the date of collection) were considered freshly overwintered if collected between 1 February and 1 May, whereas non-freshly overwintered ants were those collected between 15 May and 15 December. Ants maintained in the laboratory longer than 15 days were considered non-freshly overwintered regardless of collection date.

Monitoring gyne production. With the aid of a dissecting microscope, experimental colonies were examined weekly for the presence of female sexual (gyne) larvae (1.9–3.6 mm long) and pupae which were counted without removal. Gyne larvae are easily distinguished from worker larvae (maximum length 1.9 mm) by their size and from the large shiny male larvae by their mat appearance (Passera *et al.*, 1988b). Carbon dioxide gas was used to slow the ants while setting up the experiments and during weekly examin-

ations. Experiments were performed using one of two sizes of colony fragments. Small colony fragments consisted of c. 1200 workers, 200–300 larvae of all stages and thirty eggs, whereas large fragments contained 5000–8000 workers, several thousand pupae and larvae of all stages, and hundreds of eggs.

The data consisted of counts of the number of gyne larvae and pupae, and these were normalized by the square-root transformation (Zar, 1984) before subjecting to the appropriate parametric tests.

Results

Effect of season on gyne development

To determine whether overwintering has an effect on gyne production, small queenless colony fragments were drawn from colonies collected at various times of the year, and the number of gyne larvae and pupae present on week 4 was counted. All of these colonies were fresh from the field, i.e. were set up 7–12 days from the date of collection.

Overwintering appears to have a highly stimulatory effect on gyne production (Table 1); of the colonies tested, three of the four composed of freshly overwintered individuals produced 3–8 times more gynes than did those taken from the field at other times of the year. The remaining freshly overwintered colony, collected 6 March 1989, did not differ significantly from the non-freshly overwintered colonies. The fact that gyne larvae developed in all colonies indicates that there are some gyne-potent larvae present throughout the year.

The stimulatory effect of overwintering seems to disappear after a few weeks of summer conditions. Three colonies were collected during the freshly overwintered period but were held under summer conditions in the laboratory for 3–6 weeks before dividing into colony fragments. These units produced as few gynes as summer- or autumn-collected ants (compare Tables 1 and 2). This difference is best seen when the ants collected on 11 March 1988 are considered. The group shown in Table 2 was drawn from the same source colony as the freshly overwintered group in Table 1; after 6 weeks of summer conditions in the laboratory, it produced only one-third as many gynes.

An attempt was made to induce ants to overwinter in the laboratory. Four colonies, two collected on 11 March and two collected on 11 April 1988, were subjected to the following regime. From the date of collection, the ants were held under summer conditions (28°C and LD 16:8) until 25 July 1988 (15 and 19 weeks after collection). They were then kept for 2 weeks at 20°C and LD 12:12, before placing at 13°C and LD 8:16 for 5 weeks. The colonies were then held for 2 weeks at 20°C and LD 12:12 before returning to summer conditions. After 1 week under summer conditions, three small queenless experimental units were drawn from each colony and the number of gyne larvae and pupae was counted 4 weeks later.

As seen in Table 3, two of the artificially overwintered

Table 1. Effect of overwintering on the production of gynes in small experimental units. Shown is the number of gyne larvae and pupae (mean \pm SD) present at week 4. $F_{6,33} = 18.50$, $P < 0.0001$ (square-root transformed data).

Date collected	<i>n</i>	No. of gyne larvae and pupae	Results of the Newman-Keuls test*
Freshly overwintered			
9 February 1989	10	24.9 \pm 10.7	A
16 April 1989	5	12.4 \pm 2.3	B
11 March 1988	5	9.4 \pm 5.7	B
6 March 1989	5	2.2 \pm 1.6	C
Non-freshly overwintered			
30 July 1988	5	2.6 \pm 3.6	C
27 May 1988	5	2.4 \pm 2.6	C
12 December 1988	5	1.8 \pm 1.3	C

* Treatments with different letters differed significantly ($P < 0.05$).

groups produced relatively high numbers of gynes (eight and ten per unit). These levels were achieved only in freshly overwintered ants coming from the field, indicating that laboratory overwintering did have a stimulatory effect on some source colonies. The fact that it only occurred in groups drawn from two source colonies suggests that the regime of artificial overwintering was not as effective as natural overwintering and/or there were differences among source colonies in their sensitivity to the treatment.

Effect of overwintering on larvae and workers

This apparent stimulatory effect of overwintering could have been exerted on the larvae directly through increasing their tendency to sexualize and/or on the rearing behaviour of workers such that they are more likely to rear bipotent larvae as gynes. In the following experiment we investigated the effect of overwintering on larvae and workers by setting up small rearing units comprised of various combinations of freshly overwintered and non-freshly overwintered brood and workers. The freshly overwintered ants came from two colonies collected on 15 April 1989

and set up on 23 April. The non-freshly overwintered ants came from two colonies collected on 24 January 1989 and kept under summer conditions for 12 weeks before set up on 23 April. Four different treatments were tested: units composed of freshly overwintered brood and workers; units composed of freshly overwintered brood but non-freshly overwintered workers; those with freshly overwintered workers but non-freshly overwintered brood; and those made of non-freshly overwintered workers and brood. Construction of some treatments required mixing workers and brood from different source colonies, so this procedure was followed for all treatments in order to equalize any possible effect of combining non-nestmates.

Units made of freshly overwintered workers and brood produced far more gyne larvae and pupae than did the other treatments (Fig. 1). Nonetheless, the units containing either freshly overwintered workers or brood produced slightly more gynes than did those made of non-freshly overwintered workers and brood, suggesting that overwintering affects both the rearing behaviour of workers and the developmental potential of larvae. Apparently, the combination of the two is considerably greater than the effect of overwintering on either separately.

Table 2. Effect of laboratory summer conditions on gyne production (mean \pm SD). The small rearing units came from source colonies collected during the freshly overwintered period but were maintained in the laboratory for several weeks before the start of the experiment. The colony collected 11 March 1988 also served as the source for one of the freshly overwintered groups in Table 1. $F_{2,14} = 4.5$, $P < 0.04$ (square-root transformation).

Date collected	Days under summer conditions in laboratory before set up	<i>n</i>	No. of gyne larvae and pupae	Results of the Newman-Keuls test*
11 March 1988	41	5	3.2 \pm 2.6	A
11 April 1988	23	5	2.0 \pm 1.4	B
15 February 1988	37	5	0.2 \pm 0.4	C

* Treatments having different letters differed significantly ($P < 0.05$).

Table 3. Gyne production (mean \pm SD) in queenless test units after artificial overwintering in the laboratory. $n = 3$ in each case.

Date of collection	No. of gyne larvae and pupae
11 March 1988	0.7 \pm 1.2
11 March 1988	3.0 \pm 2.0
11 April 1988	8.0 \pm 1.0
11 April 1988	10.0 \pm 0.0

Seasonal variation in sexualization potential in relation to queen control

Because overwintering had a stimulatory effect on gyne development, it was of interest to know whether this was sufficient to overcome the inhibitory effect that queens exert on gyne production (Vargo & Passera, 1991). This was investigated by dividing stock colonies, varying in how recently they had overwintered, into three large colony fragments. These were given ten nestmate queens (polygyne treatment), one nestmate queen (monogyne treatment), or no queens (queenless treatment). The dates of collection and set-up as well as the results are given in Table 4. The fact that no gynes were produced in nearly all replicates in three of the six groups precluded the use of an analysis of variance test, normally the appropriate test for such an experimental design, because of a large inequality of variances. Therefore the two comparisons of interest were made with the appropriate *t*-tests.

In nearly all (seven of eight) cases, queenless colony fragments produced gynes. Consistent with the stimulatory effect of overwintering on gyne production, more were produced in the freshly overwintered fragments than in the non-freshly overwintered ones, although this differ-

ence was not significant ($t_6 = 1.54$, $P > 0.08$, square-root transformation). The presence of ten queens was sufficient to inhibit gyne production in both groups. Interestingly, the presence of a single queen was totally inhibitory in the non-freshly overwintered group but not in the freshly overwintered group, where some gyne larvae or pupae were present in all of the fragments. In the freshly overwintered group there were significantly fewer gynes produced in the monogyne fragments than in the queenless fragments ($t_3 = 2.73$, $P < 0.04$, paired data), suggesting that, although somewhat inhibitory, a single queen was not strong enough to overcome the stimulatory effect of overwintering. Alternatively, the inhibitory capability of individual queens may have varied with season, being lower in spring than at other times of the year.

To distinguish between these two possibilities, three pairs of large queenless colony fragments were set up from freshly overwintered ants. One fragment in each pair received a single nestmate (i.e. freshly overwintered) queen and the other received a single non-freshly overwintered (non-nestmate) queen from a stock colony held in the laboratory for several months under summer conditions. Nestmate queens were used in the former treatment and foreign queens in the latter because only a single freshly overwintered colony was available at the time each colony was divided. While this admittedly introduced the extra variable of queen source into the experiment, any possible difference in the effect of nestmate and non-nestmate queens was thought to be relatively small given that the presence of a single queen appears to have an all-or-none effect depending on season (see Table 4) and that queens can be transferred among colonies without any signs of hostility from the recipient workers (unpublished data). All queens were dissected at the end of the experiment and were found to be inseminated and have developed ovaries.

In all three cases (Table 5), gynes were produced in

Table 4. Effect of season and queen number on the production of gynes in large colony fragments. Treatments: polygyne (P), monogyne (M) and queenless (Q-).

Date collected	Days in laboratory (summer conditions) before set-up	No. of gyne larvae and pupae present after 4 weeks		
		Q-	M	P
Freshly overwintered				
9 February 1989	12	365	290	1
6 March 1989	12	327	25	0
15 April 1989	9	237	139	0
1 May 1989	7	157	1	0
Mean \pm SD		271.5 \pm 93.3	113.8 \pm 132.0	0.2 \pm 0.5
Non-freshly overwintered				
2 February 1988	90	213	0	0
2 May 1988	42	135	0	0
30 July 1988	11	0	0	0
12 December 1988	11	233	0	0
Mean \pm SD		145.3 \pm 105.7	0	0

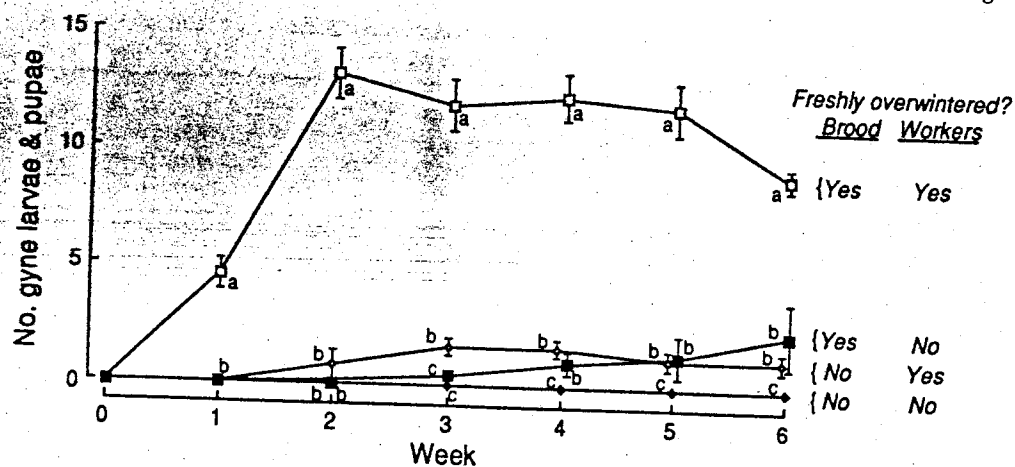


Fig. 1. Effect of overwintering on the developmental potential of female larvae and the tendency of workers to rear sexual-potent larvae as gynes. Data points are means \pm SE ($n = 5$ in each case). Differences among treatments were significant at all weeks (all $F_{3,19} \geq 33.9$, $P < 0.0001$, square-root transformed data). Treatments with different letters differed significantly at a particular week ($P < 0.05$, Newman-Keuls test).

units receiving non-freshly overwintered queens, indicating that the failure of a single queen to inhibit gyne production in these fragments was not due to a decrease in individual inhibitory capability associated with seasonal variation. This conclusion was supported by the results of a complementary experiment involving a single non-freshly overwintered colony split into three large fragments. One fragment received a freshly overwintered (non-nestmate) queen; one fragment received a non-freshly overwintered (nestmate) queen; and the final fragment was queenless. No gynes were reared in either of the fragments containing queens, whereas seventy-one gyne larvae were present on the fourth week in the queenless fragment. Again, upon dissection both queens were found to be inseminated and reproductively active.

Although the sample sizes in these experiments were very small, the data suggested that the inhibitory capability of queens did not vary much with season, whereas the sensitivity of the workers and brood to the queen inhibitory pheromone did, being much less in spring than at other times of the year.

Effect of queen age on the developmental potential of eggs

Another possible factor that may contribute to the seasonal production of gynes is an effect of queen age on the potential of their eggs to sexualize. Such a relationship has been reported for *Myrmica rubra* (Brian & Hibble, 1964) and *Monomorium pharaonis* (Petersen-Braun, 1977). If the fertilized eggs of newly mated queens were significantly less likely to develop into gynes than the eggs of older queens, this would help account for the rapid termination of gyne production after mating of the new crop of gynes. To investigate the possibility that the eggs laid by newly mated queens are incapable of developing into gynes, the following experiment was performed. Gynes were produced in small queenless rearing units. Male pupae were removed to prevent mating until the desired time. Five days after eclosion, gynes were placed in a queenless rearing unit and males (two for each female) from a foreign stock colony were introduced. Dealation (wing shedding) was taken as a sign of successful insemination (Passera *et al.*, 1988b).

Table 5. Effect of season on the inhibitory capability of queens. Workers and brood were freshly overwintered, whereas queens were either freshly overwintered or non-freshly overwintered.

Date colony collected	Days in laboratory (summer conditions) before set-up	No. of gyne larvae and pupae present after 4 weeks	
		Freshly overwintered (nestmate) queen	Non-freshly overwintered (non-nestmate) queen
9 February 1989	15	290	
15 April 1989	9	211	97
1 May 1989	7	0	277
Mean \pm SD		167 \pm 150	18
			131 \pm 133

Table 6. Ability of eggs laid by young queens to develop into gynes. There were five to ten queens in each unit for a 5-day period.

Age of queen (days)	No. of units	No. of units producing gyne larvae/pupae after 6 weeks	Mean no. of gyne larvae/pupae produced (\pm SD)
0-4	1	1	1
5-9	7	3	1.8 \pm 2.6
10-15	7	4	1.0 \pm 1.0

These newly mated queens were placed in groups of five to ten into rearing units containing 1 ml broodless workers for 5 days, after which time they were removed. Rearing units were given queens which had mated 0-4 days before ($n = 1$ unit), queens mated 5-9 days before ($n = 9$ units), or queens mated 10-14 days before ($n = 10$ units). The units were monitored weekly for 6 weeks for the presence of gyne larvae and pupae. Five units from the latter two groups were eliminated from the analysis because they had only a few larvae or abnormally high worker mortality.

The results (Table 6) show that at least some eggs produced by these young queens were able to sexualize because over half (eight of fifteen) of the units produced at least one sexual larva (mean \pm SD = 1.27 ± 1.75). Unfortunately, because the eggs of older queens were not tested in a comparable fashion, it was not possible to say whether the ability of these eggs to sexualize was equivalent or was significantly different.

Discussion

Overwintering has a strong stimulatory effect on gyne development in *I. humilis*. This is consistent with the springtime production of gynes from overwintered larvae in field colonies in southern France. Opposing this stimulatory influence is an inhibitory effect of queen control. We have recently shown (Vargo & Passera, 1991) that *I. humilis* queens produce a pheromone that inhibits gyne production, presumably by causing workers to restrict the quantity and/or quality of food given to bipotent female larvae so that their development is channeled into the worker rather than the sexual line. In addition to suppressing the sexualization of larvae, the pheromone prevents gyne production by inducing workers to execute larvae after they have undergone gyne determination.

Thus the timing and extent of gyne production in the field appear to be determined by the interplay between the stimulatory effect of winter chilling and the inhibitory influence of pheromonal queen control. Integrating these two influences with field observations on the conditions under which gynes are produced in California (Markin, 1970; Bartels, 1983) and southern France (Benois, 1973; Keller *et al.*, 1989a), we propose the following scheme for the regulation of gyne production in nature (Fig. 2). Gyne-potent larvae are present throughout the year, although

their sexualization potential (including both their developmental potential and the tendency of workers to rear them as gynes) is generally low. The development of these sexual-potent larvae as gynes is inhibited by the strong pheromonal queen control associated with high queen number (c. 10-15 queens/1000 workers). The high gyne potentiality of larvae in spring as a result of overwintering coincides with a drastic drop in queen number, and presumably the level of inhibitory queen pheromone, when the workers execute some 90% of the queens (Keller *et al.*, 1989a). The increased tendency of larvae to sexualize is sufficient to overcome the weakened inhibitory control resulting from the small number of surviving queens; consequently, there is a flush of gynes produced. Since gynes mature and mate in the nest within days of eclosion (Markin, 1970; Bartels, 1983; Passera *et al.*, 1988b), queen number is soon restored to its previously high level. These young queens begin producing the pheromone soon after mating (Vargo & Passera, 1991) thereby reinstating the inhibitory influence and preventing additional gynes from developing. This strong queen control and low sexualization ability of larvae then remain in effect until the following spring when the cycle is repeated. While the interaction of these two factors appears sufficient to account for the observed period of gyne production in the field, further investigation is needed to test this hypothesized scheme. Of particular importance will be studies of the conditions surrounding gyne production in native South American populations.

That there may be some variability in the role of these factors in different populations or climates is suggested by the observation in southern Louisiana and California (Newell & Barber, 1913; Markin, 1970) that *I. humilis* gynes appear to develop from the first eggs laid in spring rather than from overwintered larvae. Thus while overwintering increases the sexualization potential of larvae, this may not be a necessary condition for gyne production in field colonies. It is possible that the increased tendency of freshly overwintered workers to rear sexual-potent larvae as gynes is sufficient to overcome the reduced levels of inhibitory pheromone following queen execution.

The dual ability of overwintering to stimulate gyne production by affecting both the developmental potential of larvae and the rearing behaviour of workers has been demonstrated in other ants, e.g., the formicines *Plagiolepis pygmaea* (Passera, 1969), *Formica polyctena* and *F. pratensis*

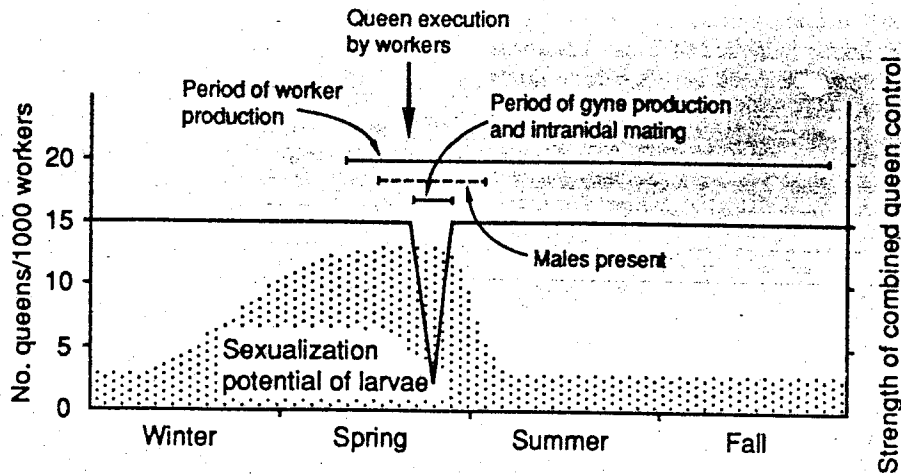


Fig. 2. Hypothesized scheme of the role of overwintering and queen control in regulating gyne production in natural populations of *I. humilis*. This scheme represents an integration of the present investigation and information coming from field studies (Markin, 1970; Benois, 1973; Bartels, 1983; Keller *et al.*, 1989a) and previous laboratory studies (Passera *et al.*, 1988a; Vargo & Passera, 1991). The potential of female larvae to sexualize (including both their developmental potential and the tendency of workers to rear them as gynes) is highest in spring following winter chilling. This high sexualization potential coincides with the brief period of gyne production which occurs just after queen number, and consequently the strength of combined queen control exerted by the colony queens, is greatly reduced by the massive execution of queens by workers.

(Gösswald & Bier, 1954b, 1957), and the myrmicine *Myrmica rubra* (Brian, 1955, 1962, 1963), and may well be a general phenomenon in species in which gyne production is strongly influenced by season. The relative weight of these two influences and their precise degree of interaction in these species, as in *I. humilis*, remain to be determined.

Little is known about the specific changes overwintering induces in larval development, but there are some indications about the larval stages affected; where it has been studied in detail, it appears that the larger larvae are most influenced. In the myrmicine *Myrmica rubra*, larvae of all stages overwinter, but only those in the third and final stadium are capable of gyne determination (Brian, 1955, 1963). A similar phenomenon occurs in the formicines *Plagiolepis pygmaea* and *Camponotus aethiops*. In *P. pygmaea* there are five larval instars; L1–L3 larvae overwinter but only the last are gyne-potent (Passera, 1969). *C. aethiops* has five larval instars in the worker and six in the queen caste. Most larvae overwinter as L1 and L2, but gynes develop from the small proportion of overwintered L5 larvae (Dartigues & Passera, 1979). The ability to sexualize in relation to larval size has not been studied systematically in *I. humilis*, but preliminary results (unpublished data) suggest that larger larvae are also most influenced by overwintering. This would help account for the relatively rapid appearance of gyne larvae following queenlessness in freshly overwintered colony fragments.

The increased ability of workers to rear gyne-potent larvae after winter chilling most probably lies in a change in the quantity and/or quality of food given to larvae. In *M. rubra*, workers in spring speed up development of larvae by feeding them more than at other times of the year (Brian, 1962). Although this additional food does not appear to affect the developmental fate of larvae, it does

ensure that gyne-potent larvae are reared to adulthood in spring and not forced to undergo a second period of overwintering. Workers of *Formica polyctena* and *F. pratensis* are especially potent at rearing gynes in spring when they feed larvae food rich in protein and oil from the pharyngeal glands; in summer this is diluted with honeydew from the crop (Gösswald & Bier, 1957). In *I. humilis* the fat content of workers is highest when they are young (Passera & Keller, 1987), but it is not known whether there are seasonal changes related to the increased larval rearing ability in spring.

In some ants, low sexualization potential of eggs laid by young queens and variation in queen inhibitory power with season and/or age contribute significantly to the regulation of gyne production. For example, in laboratory colonies of the polygyne ant, *Monomorium pharaonis*, sexuals are produced cyclically at intervals of 3–4 months (Petersen-Braun, 1975). Age dependent variation in both the sexualization potential of eggs and the inhibitory power of queens seem to underly this cycle. Young queens (<4 weeks old) lay almost exclusively worker-determined eggs (Petersen-Braun, 1977). As the queens age, the ability of their eggs to sexualize increases, but so does their ability to inhibit this sexualization. Near the end of their 3–4-month lifespan their inhibitory power wanes and a new crop of sexuals is produced (Petersen-Braun, 1975). These sexuals mate in the nest, and the dying queens are replaced with a fresh group. In *Myrmica rubra*, the eggs of younger queens have a tendency to be worker-biased (Brian & Hibble, 1964). Also, older queens have a stronger effect on larval growth than younger queens and are most influential in spring (Brian & Hibble, 1964). The situation appears to be different in *I. humilis*, as the results of the present study suggest that in this species the sexualization potential of larvae and the inhibitory power of

queens are not greatly affected by queen age and season. However, a possible minor influence of these factors on gyne determination cannot be ruled out. It will be up to future studies to see whether such effects exist and, if so, their relative roles in regulating gyne production.

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References

- Bartels, P.J. (1983) Polygyny and reproductive biology of the Argentine ant. Ph.D. dissertation, University of California, Santa Cruz.
- Benois, A. (1973) Incidence des facteurs écologiques sur le cycle annuel et l'activité saisonnière de la fourmi d'Argentine, *Iridomyrmex humilis* Mayr, dans la région d'Antibes. *Insectes Sociaux*, 20, 267–296.
- Bier, K. (1953) Beziehungen zwischen Nährzellkerngröße und Ansbildung ribonukleinsäurehaltiger Strukturen in den Oocyten von *Formica rufa rufa-pratensis minor* Gösswald. *Zoologischer Anzeiger Supplement*, 17, 369–374.
- Bier, K. (1954) Über den Saisondimorphismus der Oogenese von *Formica rufa rufa-pratensis minor* Gössw. und dessen Bedeutung für die Kastendetermination. *Biologisches Zentralblatt*, 73, 170–190.
- Brian, M.V. (1955) Studies of caste differentiation in *Myrmica rubra* L. 3. Larval dormancy, winter size and vernalisation. *Insectes Sociaux*, 2, 85–114.
- Brian, M.V. (1962) Studies of caste differentiation in *Myrmica rubra* L. 5. Social conditions affecting early larval differentiation. *Insectes Sociaux*, 9, 295–310.
- Brian, M.V. (1963) Studies of caste differentiation in *Myrmica rubra* L. 6. Factors influencing the course of female development in the early third instar. *Insectes Sociaux*, 10, 91–102.
- Brian, M.V. (1979) Caste differentiation and division of labor. *Social Insects*, Vol. I (ed. by H. R. Hermann), pp. 121–222. Academic Press, New York.
- Brian, M.V. (1980) Social control over sex and caste in bees, wasps and ants. *Biological Reviews of the Cambridge Philosophical Society*, 55, 379–415.
- Brian, M.V. & Hibble, J. (1964) Studies of caste differentiation in *Myrmica rubra* L. 7. Caste bias, queen age and influence. *Insectes Sociaux*, 11, 223–238.
- Dartigues, D. & Passera, L. (1979) Polymorphisme larvaire et chronologie de l'apparition des castes femelles chez *Camponotus aethiops* Latreille (Hymenoptera, Formicidae). *Bulletin de la Société Zoologique de France*, 104, 197–207.
- Fletcher, D.J.C. & Ross, K.G. (1985) Regulation of reproduction in eusocial Hymenoptera. *Annual Review of Entomology*, 30, 319–343.
- Gösswald, K. & Bier, K. (1953) Untersuchungen zur Kastendetermination in der Gattung *Formica*. 2. Die Aufzucht von Geschlechtstieren bei *Formica rufa pratensis* (Retz.). *Zoologischer Anzeiger*, 151, 126–134.
- Gösswald, K. & Bier, K. (1954a) Untersuchungen zur Kastendetermination in der Gattung *Formica*. 3. Die Kastendetermination von *Formica rufa pratensis minor* Gössw. *Insectes Sociaux*, 1, 229–246.
- Gösswald, K. & Bier, K. (1954b) Untersuchungen zur Kastendetermination in der Gattung *Formica*. 4. Physiologische Weisellosigkeit als Voraussetzung der Aufzucht von Geschlechtstieren im polygynen Volk. *Insectes Sociaux*, 1, 305–318.
- Gösswald, K. & Bier, K. (1957) Untersuchungen zur Kastendetermination in der Gattung *Formica*. 5. Der Einfluss der Temperatur auf die Eiablage und Geschlechtsbestimmung. *Insectes Sociaux*, 4, 335–348.
- Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. Belknap Press of Harvard University Press, Cambridge, Mass.
- Keller, L., Passera, L. & Suzzoni, J.-P. (1989a) Queen execution in the Argentine ant, *Iridomyrmex humilis*. *Physiological Entomology*, 14, 157–163.
- Keller, L., Cherix, D. & Ulloa-Chacon, P. (1989b) Description of a new artificial diet for rearing ant colonies as *Iridomyrmex humilis*, *Monomorium pharaonis* and *Wasmannia auropunctata* (Hymenoptera, Formicidae). *Insectes Sociaux*, 36, 348–352.
- Markin, G.P. (1970) The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in southern California. *Annals of the Entomological Society of America*, 63, 1238–1242.
- Newell, W. & Barber, T.C. (1913) The Argentine ant. *United States Department of Agriculture, Bureau of Entomology Bulletin No. 122*.
- Passera, L. (1969) Biologie de la reproduction chez *Plagiolepis pygmaea* Latreille et ses deux parasites sociaux *Plagiolepis grassei* Le Masne et Passera et *Plagiolepis xene* Stärcke (Hymenoptera, Formicidae). *Annales des Sciences Naturelles, Zoologie, Paris*, 12^e Série, 11, 327–482.
- Passera, L. (1980a) La fonction inhibitrice des reines de la fourmi *Plagiolepis pygmaea* Latr.: rôle des phéromones. *Insectes Sociaux*, 27, 212–225.
- Passera, L. (1980b) La ponte d'oeufs préorientés chez la fourmi *Pheidole pallidula* (Nyl.) (Hymenoptera-Formicidae). *Insectes Sociaux*, 27, 79–95.
- Passera, L. (1984) *L'Organisation Sociale des Fourmis*. Privat, Toulouse.
- Passera, L. & Keller, L. (1987) Energy investment during the differentiation of sexuals and workers in the Argentine ant *Iridomyrmex humilis* (Mayr). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, 60, 249–260.
- Passera, L., Keller, L. & Suzzoni, J.-P. (1988a) Control of brood male production in the Argentine ant *Iridomyrmex humilis* (Mayr). *Insectes Sociaux*, 35, 19–33.
- Passera, L., Keller, L. & Suzzoni, J.-P. (1988b) Queen replacement in dequeened colonies of the Argentine ant *Iridomyrmex humilis* (Mayr). *Psyche*, 95, 59–65.
- Passera, L. & Suzzoni, J.-P. (1991) Role of morphogenetic hormones in caste polymorphism in ants. In: *Morphogenetic Hormones in Arthropods* (ed. by A. P. Gupta). Rutgers University Press, New Brunswick, New Jersey.
- Petersen-Braun, M. (1975) Untersuchungen zur sozialen Organisation der Pharaoameise *Monomorium pharaonis* L. (Hymenoptera, Formicidae). I. Der Brutzyklus und seine Steuerung durch populationseigene Faktoren. *Insectes Sociaux*, 22, 269–292.
- Petersen-Braun, M. (1977) Untersuchungen zur sozialen Organisation der Pharaoameise *Monomorium pharaonis* L. (Hymenoptera, Formicidae). II. Die Kastendetermination. *Insectes Sociaux*, 24, 303–318.
- Plateaux, L. (1970) Sur le polymorphisme social de la fourmi

- Leptothorax nylanderi* (Förster). I. Morphologie et biologie comparées des castes. *Annales des Sciences Naturelles, Zoologie, Paris*, 12^e Série, 12, 373-478.
- Plateaux, L. (1971) Sur le polymorphisme social de la fourmi *Leptothorax nylanderi* (Förster). II. Activité des ouvrières et déterminisme des castes. *Annales des Sciences Naturelles, Zoologie, Paris*, 12^e Série, 13, 1-90.
- Suzzoni, J.-P., Passera, L. & Strambi, A. (1981) Ecdysteroid titre and caste determination in the ant *Pheidole pallidula* (Nyl.) (Hymenoptera: Formicidae). *Experientia*, 36, 1228-1229.
- Vargo, E.L. & Passera, L. (1991) Pheromonal and behavioral queen control over the production of gynes in the Argentine ant *Iridomyrmex humilis* (Mayr). *Behavioral Ecology and Sociobiology*, 28, 161-169.
- Wheeler, D.E. (1986) Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *American Naturalist*, 128, 13-34.
- Wilson, E.O. (1971) *The Insect Societies*. Belknap Press of Harvard University Press, Cambridge, Mass.
- Zar, J.H. (1984) *Biostatistical Analysis*, 2nd edn. Prentice-Hall, Englewood Cliffs, New Jersey.

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