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# Influence of insect hormones on the establishment of dominance hierarchies among foundresses of the paper wasp, *Polistes gallicus*

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**Summary.** Laboratory studies on overwintered foundresses of *Polistes gallicus* show that differences in the endocrine activity are mainly responsible for achieving the dominance rank. Females that became dominant had either larger corpora allata or more developed ovaries than subordinate females. Body size did not contribute to dominance rank. Since a correlation exists between the length of terminal oocytes and the ecdysteroid titre in haemolymph as well as between the volume of corpora allata and the synthesis of juvenile hormone, dominant behaviour is thought to depend upon an elevated hormone titre in haemolymph. Injections of juvenile hormone (JHI) and 20-hydroxyecdysone, separately and simultaneously, significantly increased the probability that the treated female would be the dominant female of a test pair. After a hierarchy has been established, endocrine activity in subordinate foundresses is inhibited by the dominant foundress that then monopolizes reproduction.

## Introduction

In the paper wasp, *Polistes gallicus*, spring nests are started by one to several hibernated foundresses (Heldmann 1936; Pardi 1948). After a period of frequent dominant-subordinate interactions females form a linear dominance hierarchy. One of the foundresses becomes the principal egg-layer. The subordinate foundresses function as workers; they do not reproduce as long as the dominant female is present (Pardi 1946, 1948). If they lay eggs, which they sometimes do during early nest initiation, the eggs are eaten by the dominant female ("differential oophagy", Gervet 1964).

Dominant females are sometimes larger and have better developed ovaries than subordinate females (Pardi 1946, 1948; Gervet 1962; Turillazzi and Pardi 1977; Dropkin and Gamboa 1981 for *Polistes metricus*; Noonan 1981 for *Polistes fuscatus*). Previous investigations have shown that dominant foundresses also have enlarged corpora allata (CA) and that this enlargement is correlated with an increased synthesis of juvenile hormone (JH) (Pardi 1980; Röseler et al. 1980). The relationship between dominant behaviour and CA activity was confirmed by Turillazzi et al. (1982) for *Polistes gallicus* and documented in short abstracts by Ross and Gamboa (1982) for *Polistes metricus* and by Vawter and Strassmann (1982) for an unnamed species (*Polistes annularis*?).

These results provide strong support for the hypothesis that high dominance rank is based on high endocrine activity and high reproductive capacity. But we could not decide whether increased endocrine activity is a cause of dominant behaviour or the consequence of high rank, since all previous investigations were done days or even weeks after the dominance hierarchy had been established. Therefore, in the present study we investigated whether differences in the activity of CA and oocyte development are responsible for becoming a dominant foundress, and whether the endocrine activity will be suppressed in a female when becoming subordinate. To understand better the factors governing dominant behaviour we determined whether the injection of JH and 20-hydroxyecdysone enhances the probability that a female will become dominant. Preliminary results were published in a short communication (Röseler et al. 1981).

## Materials and methods

*Wasps. Polistes gallicus* females were collected from their hibernating sites near Marseille and thereafter transferred into small plastic boxes (10 × 10 × 10 cm, filled with soft paper and a water source) and kept at 15 °C in dim daylight until used. The experiments were carried out in the laboratories of CNRS in Marseille and in the Zoological Institute in Würzburg.

*Test for dominance.* Behavioural observations were always conducted at the same time of day. Females were taken out of the cold room in the morning, individually marked, and separated into plastic cages (10 × 10 × 10 cm) supplied with water and honey. The cages were kept at room temperature (approx. 20 °C) in daylight. After 24 h the cages were heated to about 30 °C by a lamp for 1 h. Thereafter the tests were carried out as follows: Two females were allowed to enter a new cage. As a rule, agonistic behaviour is shown by them both at the very first meeting. The females started antennating each other, and then one tried to make the other akinetic by climbing on it with its forelegs, by biting and mock stinging it, finally by chewing with its mandibles above the other. Immediately after dominance was established, normally within a few minutes, the females were separated, killed, and the activity of the ovaries and CA measured using techniques detailed below.

*Body size.* We used the length of cubital cell 1 of the forewing as an index of size. We measured the length under a microscope with an ocular micrometer; it is expressed as mm wing-index.

*Development of ovaries.* The ovaries were dissected in Ringer's solution, and the length of all six terminal oocytes was measured under a microscope with an ocular micrometer. The mean value was used as an index of ovarian activity.

*Volume of CA.* The CA were dissected in Ringer's solution, the adherent tissue was removed, and the glands were transferred into a blood cell counting chamber (depth 0.1 mm) containing a droplet of Ringer's solution. The planes of both glands were drawn using drawing equipment for microscopes (Zeiss), and the volume calculated from the plane and the depth of the chamber. The average value of both glands was used.

*Determination of ecdysteroid titre.* We shut the mouthparts and the tip of the abdomen of the wasps with paraffin, made a small dorsal incision in the thorax, and then put the wasps head foremost into pipette tips which were placed into vials. After slow centrifugation the haemolymph was collected, the volume measured, the threefold amount of 80% methanol added, thoroughly mixed by sonication, and incubated at 40 °C for 1 h. Thereafter the samples were centrifuged (6000 rev. 10 min). A known volume of methanol extract was transferred into an Eppendorf tube and evaporated to dryness at 40 °C. The ecdysteroid titres were determined by the equilibrium method (de Reggi et al. 1975). Dry extracts were dissolved in 50 µl

**Table 1.** Intercorrelation between differences in body size, CA volume, and oocyte length in untreated foundresses. *n* pairs of wasps

| | Differences in body size (mm wing-index) | | | | |
|---|---|---|---|---|---|
| | Total | <0.16 | 0.16–0.30 | 0.31–0.45 | >0.45 |
| *n* | 173 | 80 | 60 | 27 | 6 |
| $r_{\text{body-size CA-vol.}}$ constant: oocytes | 0.133 | −0.077 | −0.016 | 0.396[a] | 0.731 |
| $r_{\text{body-size oocytes}}$ constant: CA-volume | 0.112 | −0.004 | 0.010 | 0.495[b] | −0.092 |

| | Differences in CA volume ($\times 10^4$ µm$^3$) | | | | |
|---|---|---|---|---|---|
| | Total | <51 | 51–100 | 101–150 | >150 |
| *n* | 173 | 41 | 54 | 32 | 46 |
| $r_{\text{CA-vol. body-size}}$ constant: oocytes | 0.133 | −0.031 | −0.187 | 0.371[a] | 0.216 |
| $r_{\text{CA-vol. oocytes}}$ constant: body-size | 0.628[c] | 0.317[a] | 0.556[c] | 0.601[c] | 0.777[c] |

| | Differences in oocyte length (mm) | | | | |
|---|---|---|---|---|---|
| | Total | <0.11 | 0.11–0.20 | 0.21–0.30 | >0.30 |
| *n* | 173 | 84 | 63 | 21 | 5 |
| $r_{\text{oocytes body-size}}$ constant: CA-volume | 0.112 | −0.031 | 0.194 | −0.024 | 0.656 |
| $r_{\text{oocytes CA-volume}}$ constant: body-size | 0.628[c] | 0.553[c] | 0.695[c] | 0.745[c] | 0.930 |

[a] $P<0.05$
[b] $P<0.01$
[c] $P<0.001$

citrate buffer (0.1 M; pH 6.1), mixed with $^{125}$J labelled derivative and dialysed against antibody for 24 h. The ecdysteroid titre is expressed as pmol ecdysteroids/ml haemolymph.

*Injection of hormones.* We injected the hormones immediately after removing the wasps from the cold room. The wasps received 5 μg juvenile hormone (JHI) dissolved in 2 μl triolein or 40 ng 20-hydroxyecdysone dissolved in 2 μl methanol/Ringer's solution 1:200 (v/v) (both hormones were purchased from Sigma). For the injection of both hormones together we injected the same dosage, but each hormone was dissolved only in 1 μl solvent. Both hormones were drawn into the same syringe one after another and then injected. The controls received only the solvents. We injected the hormones or the solvents into the abdomen through the intersegmental membrane under the fourth tergite.

*Evaluation.* We evaluated the dominance interaction among pairs of wasps and measured the differences in the three parameters "body size", "volume of CA", and "length of oocytes". Partial correlation was used to examine the intercorrelation between the three measurements and their independent effects on dominant behaviour. Statistical significance follows Fisher (1954).

## Results

### *Dominance in untreated foundresses*

We tested dominant-subordinate interactions between untreated foundresses at their first meeting 1 day after hibernation and evaluated the independent contribution of differences in body size, CA volume, and ovary development of each pair to dominance rank. Table 1 shows the intercorrelation between the three measurements; each was additionally divided into four groups. In general, there is no significant correlation between differences in body size and differences in CA volume ($r=0.13$) or in oocyte length ($r=0.11$). But the larger the size difference between two wasps becomes, the stronger is the correlation between differences in the activity of both organs. Larger wasps were more likely to have active CA and ovaries. In contrast, differences in CA volume and

**Table 2.** Influence of differences in body size, CA volume, and oocyte length on dominance rank of foundresses and partial correlation between dominance and the independent effects of the three measurements. $n$ = pairs of wasps

| | Differences in body size (mm wing-index) | | | | |
|---|---|---|---|---|---|
| | Total | <0.16 | 0.16–0.30 | 0.31–0.45 | >0.45 |
| $n$ | 173 | 80 | 60 | 27 | 6 |
| Percentage of dominants with larger size | 77 | 52 | 50 | 81 | 83 |
| $r_{\text{dominance body-size}}$ constant: CA-volume oocytes | −0.01 | 0.018 | 0.030 | 0.006 | 0.008 |

| | Differences in CA volume ($\times 10^4$ μm$^3$) | | | | |
|---|---|---|---|---|---|
| | Total | <51 | 51–100 | 101–150 | >150 |
| $n$ | 173 | 41 | 54 | 32 | 46 |
| Percentage of dominants with larger CA volume | 89 | 80 | 85 | 91 | 100 |
| $r_{\text{dominance CA-volume}}$ constant: body-size oocytes | 0.504[c] | 0.497[c] | 0.490[c] | 0.800[c] | 0.753[c] |

| | Differences in oocyte length (mm) | | | | |
|---|---|---|---|---|---|
| | Total | <0.11 | 0.11–0.20 | 0.21–0.30 | >0.30 |
| $n$ | 173 | 84 | 63 | 21 | 5 |
| Percentage of dominants with larger oocytes | 85 | 81 | 86 | 95 | 100 |
| $r_{\text{dominance oocytes}}$ constant: body-size CA-volume | 0.351[c] | 0.381[c] | 0.435[c] | 0.768[c] | 0.951 |

[a] $P<0.05$
[b] $P<0.01$
[c] $P<0.001$

in oocyte length are significantly intercorrelated ($r = 0.628$), even if only small differences exist.

Dominant behaviour is closely related to an elevated activity of CA and ovaries (Table 2). Small differences in the activity of both organs significantly contribute to dominance position. The larger the differences are, the more likely it is that the female with larger CA or oocytes becomes dominant. The partial correlations show that body size does not influence dominant behaviour. The probability of achieving dominance was found to be increased up to 80% in larger females, but that is due to the connection between large differences in body size and differences in the activity of CA and ovaries shown in Table 1.

As some of the wasps ($n = 45$) exhibited no clear agonistic behaviour, we were unable to rank them and, therefore, could not take them into consideration. Females with CA smaller than $220 \times 10^4$ µm$^3$ did not become dominant. Wasps parasitized by the strepsipteran *Xenos vesparum* Rossi belonged to the group of non-aggressive females. Their mean CA volume was $199 \times 10^4$ µm$^3$ ($n = 19$). Though some of the parasitized females had CA larger than $220 \times 10^4$ µm$^3$, they also showed no dominant behaviour.

### *Ecdysteroid titre in haemolymph*

The results from untreated individuals indicate that females which become dominant at the first interaction after hibernation have larger CA and/or larger oocytes than their opponents. It is, therefore, very likely that dominant females have an increased endocrine activity, and Röseler et al. (1980) found a correlation between CA size and JH synthesis at that phase. Since ovaries with growing oocytes produce ecdysteroids (Strambi et al. 1977), we determined whether the ecdysteroid level in wasps is increased 1 day after the end of overwintering (Fig. 1). Even in wasps with small oocytes, ecdysteroids can be detected in the haemolymph. The rise in the level of ecdysteroids coincides with the enlargement of oocytes ($r = 0.79$; $P < 0.001$) so that the length of oocytes can be used as an index of ecdysteroid level.

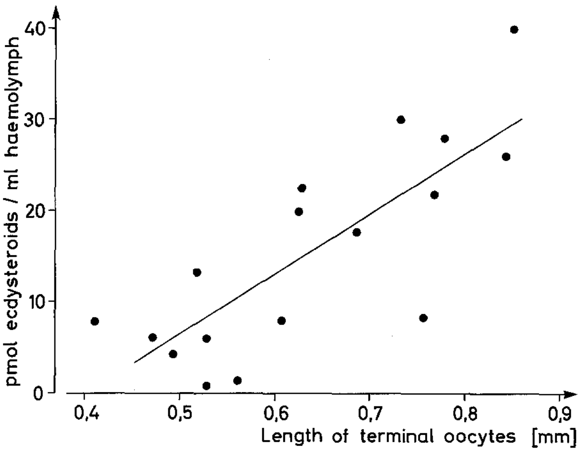

**Fig. 1.** Relationship between length of terminal oocytes and ecdysteroid titre in haemolymph ($r = 0.79$) in overwintered foundresses of *Polistes gallicus*

### *Injection of hormones*

To study the influence of hormones on dominant behaviour, we injected JHI, 20-hydroxyecdysone or both hormones simultaneously and tested the hormone-treated females against controls which only received the solvent. When we took females out of the cold room, we did not know their endocrine activity, i.e. we injected the hormones into females with different sized CA and oocytes. If the injection of hormones influences dominant behaviour, the percentage of females which become dominant in spite of a low activity of their CA and ovaries should be increased. Consequently, the dominance rank should become more or less independent of the endocrine activity, although there were, of course, hormone-treated wasps with larger CA and oocytes than the controls. On the other hand, in all couples in which the hormone-treated female becomes the subordinate, there should be a high correlation between dominance rank of the control female and its endocrine activity.

Although we have shown that dominance is independent of body size, we again examined in all experiments a possible effect of size to ensure that there was also no correlation of size with dominant behaviour in other samples. All three measurements are divided into two groups to show possible different effects of small and large differences among the test pairs.

It is, of course, essential that individuals used for the experiments were nearly the same size, and that injection of hormones did not influence the volume of CA and the length of oocytes during the 24 h until the females were tested. The mean values of the three measurements averaged over controls and over hormone-treated individuals are given in Table 3. There is no significant difference in the mean values between females which received hormones and those which received only the solvents ($t$-test).

**Table 3.** Body size, CA volume, and oocyte length of hormone-treated foundresses and of controls which received the solvents. Values are means ± standard deviation

| | Injection of | | Injection of | | Injection of | |
|---|---|---|---|---|---|---|
| | JH ($n=46$) | Solvent ($n=42$) | 20-hydroxyecdysone ($n=59$) | Solvent ($n=58$) | JH + 20-hydroxyecdysone ($n=42$) | Solvent ($n=43$) |
| Body size (mm wing-index) | 2.47 ± 0.12 | 2.53 ± 0.11 | 2.47 ± 0.2 | 2.55 ± 0.18 | 2.40 ± 0.13 | 2.44 ± 0.12 |
| CA volume ($\times 10^4$ $\mu m^3$) | 259 ± 55 | 282 ± 62 | 249 ± 72 | 257 ± 69 | 252 ± 72 | 258 ± 62 |
| Oocyte length (mm) | 0.59 ± 0.11 | 0.60 ± 0.17 | 0.54 ± 0.12 | 0.56 ± 0.15 | 0.49 ± 0.06 | 0.46 ± 0.07 |

**Table 4.** Influence of JH on dominance of foundresses. Partial correlation between dominance and the independent effects of body size, CA volume, and oocyte length. *n* pairs of wasps

| | The JH-treated foundress became | | | | | |
|---|---|---|---|---|---|---|
| | Total | Dominant | | Total | Subordinate | |
| Differences in body size (mm wing-index) | | ≤0.3 | >0.3 | | ≤0.3 | >0.3 |
| *n* | 69 | 60 | 9 | 47 | 40 | 7 |
| $r_{\text{dominance body-size}}$ constant: CA-volume oocytes | $-0.314^{b}$ | $-0.267^{a}$ | $-0.530$ | 0.016 | 0.044 | $0.937^{c}$ |
| Differences in CA volume ($\times 10^4$ $\mu m^3$) | | ≤100 | >100 | | ≤100 | >100 |
| *n* | 69 | 54 | 15 | 47 | 35 | 12 |
| $r_{\text{dominance CA-volume}}$ constant: body-size oocytes | 0.176 | 0.091 | 0.402 | $0.646^{c}$ | $0.749^{c}$ | $0.826^{c}$ |
| Differences in oocyte length (mm) | | ≤0.2 | >0.2 | | ≤0.2 | >0.2 |
| *n* | 69 | 51 | 18 | 47 | 36 | 11 |
| $r_{\text{dominance oocytes}}$ constant: body-size CA-volume | 0.085 | $0.319^{a}$ | 0.380 | 0.122 | $0.327^{a}$ | $-0.158$ |

[a] $P<0.05$ [b] $P<0.01$ [c] $P<0.001$

### *Injection of JHI (Table 4)*

In 69 pairs the JH-injected female became the dominant. There is no significant correlation between that rank and differences in size and CA volume. Only the length of oocytes in one group is moderately correlated (5% level) to dominance indicating that in these females the ovarian activity could have additionally influenced the dominant behaviour. But the injected JH is more likely to have contributed to dominance rank than the endogenous endocrine activity.

Only in 47 couples did the JH-treated wasps become subordinates; the dominant position of the control females is highly correlated to larger CA. Ovary development did not influence the behaviour to the same extent. Interestingly, in seven wasps large differences in body size also contributed to dominance position.

### *Injection of 20-hydroxyecdysone (Table 5)*

After injection of 20-hydroxyecdysone 94 females became dominant. The partial correlation shows that neither differences in body size nor differences

**Table 5.** Influence of 20-hydroxyecdysone on dominance of foundresses. Partial correlation between dominance and the independent effects of body size, CA volume, and oocyte length. *n* pairs of wasps

| | The 20-hydroxyecdysone-treated foundress became | | | | | |
|---|---|---|---|---|---|---|
| | | Dominant | | | Subordinate | |
| | Total | | | Total | | |
| Differences in body size (mm wing-index) | | $\leq 0.3$ | $>0.3$ | | $\leq 0.3$ | $>0.3$ |
| *n* | 94 | 81 | 13 | 55 | 45 | 10 |
| $r_{\text{dominance body-size}}$ constant: CA-volume oocytes | $-0.541^{c}$ | $-0.356^{b}$ | $-0.992^{c}$ | 0.176 | 0.074 | $0.678^{a}$ |
| Differences in CA volume ($\times 10^4$ $\mu m^3$) | | $\leq 100$ | $>100$ | | $\leq 100$ | $>100$ |
| *n* | 94 | 70 | 24 | 55 | 43 | 12 |
| $r_{\text{dominance CA-volume}}$ constant: body-size oocytes | $0.224^{a}$ | 0.177 | 0.386 | $0.405^{b}$ | $0.361^{a}$ | $0.857^{c}$ |
| Differences in oocyte length (mm) | | $\leq 0.2$ | $>0.2$ | | $\leq 0.2$ | $>0.2$ |
| *n* | 94 | 87 | 7 | 55 | 41 | 14 |
| $r_{\text{dominance oocytes}}$ constant: body-size CA-volume | 0.189 | 0.176 | 0.295 | $0.338^{a}$ | $-0.107$ | $0.912^{c}$ |

[a] $P<0.05$
[b] $P<0.01$
[c] $P<0.001$

**Table 6.** Influence of JH and 20-hydroxyecdysone on dominance of foundresses. Partial correlation between dominance and the independent effects of body size, CA volume, and oocyte length. *n* pairs of wasps

| | The hormone-treated foundress became | | | | | |
|---|---|---|---|---|---|---|
| | | Dominant | | | Subordinate | |
| | Total | | | Total | | |
| Differences in body size (mm wing-index) | | $\leq 0.3$ | $>0.3$ | | $\leq 0.3$ | $>0.3$ |
| *n* | 75 | 69 | 6 | 37 | 33 | 4 |
| $r_{\text{dominance body-size}}$ constant: CA-volume oocytes | 0.063 | 0.002 | $-0.015$ | $0.497^{b}$ | $0.360^{a}$ | 0.999 |
| Differences in CA volume ($\times 10^4$ $\mu m^3$) | | $\leq 100$ | $>100$ | | $\leq 100$ | $>100$ |
| *n* | 75 | 58 | 17 | 37 | 31 | 6 |
| $r_{\text{dominance CA-volume}}$ constant: body-size oocytes | 0.084 | 0.140 | 0.001 | $0.706^{c}$ | $0.641^{c}$ | $0.983^{b}$ |
| Differences in oocyte length [mm] | | $\leq 0.2$ | $>0.2$ | | $\leq 0.2$ | $>0.2$ |
| *n* | 75 | 71 | 4 | 37 | 36 | 1 |
| $r_{\text{dominance oocytes}}$ constant: body-size CA-volume | $0.494^{c}$ | $0.530^{c}$ | 0.984 | 0.258 | 0.268 | – |

[a] $P<0.05$
[b] $P<0.01$
[c] $P<0.001$

in the activity of CA and ovaries contributed to dominance rank. Body size is negatively correlated indicating that small wasps became dominant more often.

The injection of 20 hydroxyecdysone significantly promoted dominant behaviour, since only 55 hormone-treated wasps became subordinates ($\chi^2$-test, $P<0.05$). The dominance of the control females is highly correlated with an increased endocrine activity.

### *Simultaneous injection of JHI and 20-hydroxyecdysone (Table 6)*

In 75 pairs the hormone-treated females became dominant independent of differences in CA activity and body size. But the significant correlation to oocyte length indicates that ovary development could have contributed to dominance rank.

After injection of both hormones only 37 foundresses became subordinates which is significant ($\chi^2$-test, $P<0.05$). The dominance rank of the controls is based upon a high CA activity and far less upon oocyte development.

### *Comparison of the effects of different hormonal treatment*

After injection of hormones the $\alpha$-position became independent of endocrine activity. The exogenous hormones, therefore, can balance the disadvantage of a low endogenous hormone level. In Table 7 the effects of the different hormone treatments are compared. Only wasps are considered which became dominant though they had less active CA and ovaries than the subordinates. In untreated wasps we found a total of 133 females which had that double disadvantage. Only six of them (5%) became dominant in the first interaction. By injection of hormones the percentage of success could be clearly increased in all groups ($P<0.001$). But there was no significant difference in the effects of the three treatments.

### *Inhibition of CA activity*

In multiple foundress associations the subordinate females have smaller CA than the dominant females. Above we have shown that females which become dominant have larger and thus more active CA than the others, but it is not clear whether the activity of the glands is additionally inhibited by the presence of a dominant female. To investigate this problem we performed the following experiments: 6–8 wasps from the cold room were kept separately in small cages for 1 day and the next day dominance was tested. In this way a linear hierarchy was established within a group. Thereafter the low ranked females were killed and the volume of CA was estimated. The high ranked females were left together in the cage which was heated to 30 °C during day-time and supplied with water, honey, and small crickets. In seven experiments we left the $\alpha$- and the $\beta$-female together, in ten experiments the $\alpha$, $\beta$-, and $\gamma$-females.

**Table 7.** Influence of hormone injection on dominance of foundresses with smaller CA together with smaller oocytes than the opponents

| | Number of foundresses | Number of dominants | % |
|---|---|---|---|
| Untreated controls | 133 | 6 | 5 |
| Injection of JH | 44 | 11 | 25[a] |
| Injection of 20-hydroxyecdysone | 48 | 17 | 35[a] |
| Injection of JH and 20-hydroxyecdysone | 26 | 9 | 35[a] |

[a] $P<0.001$, $\chi^2$-test

**Table 8.** CA volume in foundress associations in relation to social rank. Values are means ± standard deviation

| CA volume [× $10^4$ μm$^3$] | | CA volume [× $10^4$ μm$^3$] | |
|---|---|---|---|
| 1 day after hibernation | $n$ | 7 days after hibernation | $n$ |
| $\alpha$ ? | | $\alpha$ 434 ± 123 | 7 |
| $\beta$ ? | | $\beta$ 289 ± 110 | 7 |
| $\gamma$ 334 ± 42 | 7 | | |
| $\delta$ 289 ± 84 | 7 | | |
| $\varepsilon$ 264 ± 66 | 7 | | |
| $\eta$ 215 ± 31 | 6 | | |
| $\zeta$ 115 | 2 | | |
| $\alpha$ ? | | $\alpha$ 501 ± 89 | 10 |
| $\beta$ ? | | $\beta$ 437 ± 113 | 10 |
| $\gamma$ ? | | $\gamma$ 307 ± 61 | 10 |
| $\delta$ 374 ± 38 | 10 | | |
| $\varepsilon$ 340 ± 43 | 10 | | |
| $\eta$ 292 ± 38 | 10 | | |
| $\zeta$ 283 ± 54 | 5 | | |
| $\theta$ 227 ± 60 | 5 | | |

After 6 days the wasps were killed and the sizes of CA compared to those of the subordinate females investigated 6 days before. One would expect 89% of the $\beta$-females of the two foundress groups and of the $\gamma$-females of the three foundress groups to have larger CA than the females formerly subordinate to them ($\gamma$ and $\delta$). But in two foundress groups only two of seven $\beta$-females had larger CA than the $\gamma$-females (29%) and in three foundress groups only one of ten $\gamma$-females had larger CA than the $\delta$-female (10%). This result is significantly different from the control ($\chi^2$-test, $P < 0.001$).

The mean CA volumes of all groups are listed in Table 8. The values also show the inhibition of CA of $\beta$- and $\gamma$-females. During the 6 days a divergent development took place: In subordinate females the volume of CA decreased, whereas in dominant females the size of the glands enlarged. Interestingly, there is a difference between both experimental groups. The CA of $\alpha$-females are larger in three foundress groups than in two foundress groups and also the CA of $\beta$-females are larger in three foundress groups than the CA of $\beta$-females in two foundress groups. It seems that the endocrine activity of females at the end of a hierarchy is mostly inhibited, so that a $\beta$-female of a multiple foundress association can have larger CA than a $\beta$-female of a two foundress group. These tendencies, however, are not statistically significant.

## Discussion

Dominance rank in *Polistes gallicus* is correlated with increased CA activity, elevated JH titre in haemolymph, and rapidly developing ovaries. Ovaries with growing oocytes produce ecdysteroids, the titre of that hormone markedly increases even during the early stages of oogenesis after hibernation. There is a correlation between the size of terminal oocytes and the ecdysteroid titre in haemolymph so that the length of oocytes can serve as an index of ecdysteroid production; the volume of CA can be used as an index of JH synthesis (Röseler et al. 1980). The dominant foundress, therefore, is characterized by an increased JH- and ecdysteroid titre in its haemolymph.

The results show that differences in the endocrine activity on the first day following hibernation are responsible for ranking foundresses in dominance hierarchies. The increased activity of CA and a stimulated oogenesis helps a female to become dominant. Body size does not significantly contribute to dominance. The influence of body size on dominant behaviour has been reported for several species (Turillazzi and Pardi 1977; Noonan 1981; Dropkin and Gamboa 1981) and is mainly due to a relation between large size and elevated endocrine activity. The injection of JH and/or 20-hydroxyecdysone increases the probability of success for a foundress in agonistic interactions when its own endocrine activity is low. Hormone-treated females only became subordinates when control females showed high endocrine activity.

Dominant behaviour is likely to be connected to a certain level of endocrine activity. The very small CA of females which showed no dominant interactions seemed to produce insufficient amounts of JH to elicit the behaviour. To confirm this we need to know the hormonal threshold for inducing dominant behaviour. On the other hand, it is possible that hormones have to act on the neural centres for more than the 24 h used in our experiments for inducing the full response, since the hormone treatment could not completely balance the disadvantage of very small CA and oocytes, especially in parasitized wasps.

The influence of JH on dominant behaviour has been shown by Barth et al. (1975) in *Polistes annularis*. After hormone treatment the frequency of social interactions in nests rose sharply. But whether the behavioural changes were directly caused by JH or by a disruption of social hierarchy could not be decided by the authors. Vawter and Strassmann (1982) reported that a correlation exists between JH production by CA and aggressive behaviour in *Polistes*. In honeybee workers, JH treatment, however, seems to inhibit aggressive behaviour, although a relationship exists between the size of CA and that behaviour (Breed 1982). Since Breed concluded that development of CA is inhibited by exogenous JH which itself does not induce the behaviour, the CA in bees must produce other factors regulating aggressive behaviour.

At present we cannot decide whether both hormones contribute to dominant behaviour to the same extent 1 day after hibernation. It is, for instance, possible that JH does not induce the behaviour, but stimulates the ovaries to produce ecdysteroids which elicit the dominant behaviour. It seems also likely that JH triggers the dominant behaviour, and dominance might be mainly regulated by ecdysteroids only when developed oocytes are present. The ecdysteroid titre in haemolymph increases about 100-fold in egg-laying wasps (Strambi et al. 1977), whereas the volume of CA decreases after oviposition has started (Strambi 1969). A decrease in CA volume has been also observed by Turillazzi et al. (1982) in nests with a single foundress or with two foundresses during 4 weeks after nest foundation. The possible regulation of dominant behaviour by ecdysteroid level in haemolymph is consistent with the general observation that dominance is correlated with developed ovaries (Pardi 1946, 1948 for *Polistes;* Brothers and Michener 1974; Breed et al. 1978 for the halictine bee *Lasioglossum zephyrum*; Free 1955; Röseler and Röseler 1977; van Honk et al. 1981 for bumblebees). In honeybees, aggressive workers have also developed ovaries, although to a lesser degree than egg-laying workers (Velthuis 1976). An influence of ecdysteroids on dominant behaviour is not in contradiction to the results of Deleurance (1948) who could not observe an influence of ovariectomy on social behaviour in *Polistes biglumis bimaculatus;* he, however, investigated workers ovariectomized during the first 10 days after emergence.

A dominant foundress is able to inhibit the endocrine activity in subordinate females. The activity of CA of $\beta$-females in two foundress associations was more inhibited than in groups with three foundresses. Similar results were obtained by Turillazzi et al. (1982) on nests of free-living wasps 1 and 4 weeks after nest initiation. The basis for the different development is seen in the fact that dominant females in multiple foundress associations seldom leave the nest and, moreover, that they take advantage of trophallaxis. Therefore, females with subordinates show a higher endocrine activity and ovary development than single foundresses or females at the end of a hierarchy, which cannot exploit subordinates. Our results show that differences in the endocrine activity exist in foundress associations in captivity even prior to nest initiation. At that stage only a trophic advantage for dominant females could contribute to the different development, though in cages the dominant females mainly collect their food themselves. Therefore, behavioural aspects should also be considered. The $\beta$-female in a three foundress association probably experiences less subordination, because the domination by the $\alpha$-female is shared by all subdominant females, whereas the $\gamma$-female experiences the domination by the $\alpha$- and the $\beta$-female. However, we have not made quantitative studies on behaviour. In summary, it is very likely that different factors contribute to the inhibition of endocrine activity and reproduction. Pheromones seem not to be involved in subordination, since their concentration should be the same in associations with two or three foundresses and should primarily affect the $\beta$-female which is nearest to the $\alpha$-female.

At the end of hibernation differences in the endocrine activity even exist between the foundresses from the same hibernation site (Röseler 1984). The small differences in the beginning become more and more pronounced during colony development by inhibition of subordinate females as well as by the trophic advantage and lessened external activities of the $\alpha$-female. This system originally postulated by Pardi (1946) for ovary development ensures the reproductive exclusiveness of the $\alpha$-female.

The mechanism for sorting foundresses in dominance is based upon differences in the endocrine activity. That relation offers the advantage that only those foundresses become dominant which show a high endocrine activity and with it indicate the highest possible reproductive capacity for the association (West 1967).

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