HAEMOLYMPH PROTEIN PATTERN OF DIFFERENT FORMICA CASTES DURING DEVELOPMENT

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The development of a female dimorphism is one of the most striking phenomena in the study of social Hymenoptera. In Formica a caste specific development of the imaginal organs could be shown (Schmidt 1966). According to Maidhof (1968) this is induced after the first larval instar. This developmental-physiological problem can only be understood at a molecular level. At a definite time in larval development a short, unstable period is present, in which it is possible to influence the genetic code by nutritional factors, so producing either queens or workers. In forest ants I have shown that these interactions result in considerable differences in the storage of lipids and carbohydrates by the larvae (Schmidt 1967) and therefore suggesting that differences of enzyme relations should be present.

The chemical basis of morphogenesis is a problem of differential activation of genes. According to our present knowledge about the transfer of gene information, the chemical structure of the protein molecules is the first definite product of a gene activation. Synthesis and use of haemolymph proteins are strongly controlled by genes and hormones, and they perform a specific function in the synthesis of the imaginal organs (Egelhaaf 1963, Munn and Greville 1969). Therefore the haemolymph proteins would appear to be particularly important in the investigation and interpretation of differentiated gene activation, resulting in large morphological differences between castes, even in ants. Two questions are foremost: is it possible to demonstrate caste-specific influences in protein synthesis? Does the protein pattern change differently in both castes during the metamorphosis?

Brunnert (1967) has provided the first study on caste-specific changes in the protein pattern of forest ants by starch gel electrophoresis. For our more detailed investigations we have used an improved disc-electrophoretic technique, allowing up to 21 fractions of haemolymph proteins to be separated (fig.). The width and coloration of the bands with aniline black was very different. For our investigations we have used various developmental stages of Formica polyctena Foerst; F. pratensis Retz. and F. rufa L., from several populations collected near Würzburg. The proteins of the haemolymph were directly separated on polyacrylamid gels and, after staining, measured by a Chromoscan densitometer. The single fractions were calculated as bovine serum albumin (method according to Schmidt & Hess 1973). In most pherograms only 7-8 fractions were stained strongly enough to make a quantitative determination possible, although good resolution was present. Clear differences could be demonstrated in species, localities and developmental stages.

However, in all three species, the protein pattern changes specially in the castes during metamorphosis. The slowly running protein fractions increased a little in the blood of prepupae of queens but were reduced during metamorphosis, as in workers, reflecting the different

development of the imaginal organs (Schmidt 1967). During metamorphosis, the relationship of the quickly running fractions to the slow ones increased, favouring the former. Apart from species-specific differences, the single fractions were reduced in both castes in a definite manner. In consequence of this caste-specific reduction, there were significant differences in the protein pattern of the castes during the later developmental stages. But these were only quantitative. In one of the two castes certain bands were reduced so strongly that it was not possible to recognise them in the pherogram, except as an increased haemolymph volume. For the interpretation of these caste differences it is therefore vital to extend such investigations over the whole developmental period.

To answer the question of whether the protein synthesis will be influenced directly by the effect of determination, and whether the observed differences of the protein pattern are dependent only on the caste specific organ development, the protein fractions were compared before organogenesis. Definite fractions can be observed at the end of the larval development and therefore spinning larvae or prepupae were used as definitive stages for the comparison of the castes. At this time the most complete protein pattern is present, as synthesised during the larval period. Furthermore, only developmental stages from the same social unit are appropriate for a caste comparison.

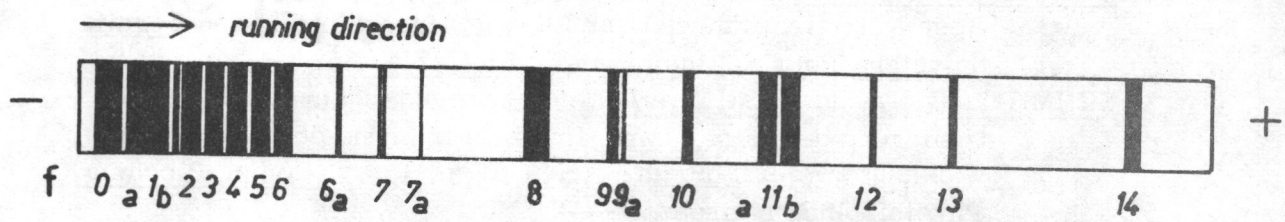
Considering the possibilities of error, significant differences take place in the protein pattern of the haemolymph of both female castes in spite of the important differences found between different populations. Some of the variations are demonstrated as follows.

Formica pratensis prepupae from Randersacker (monogynous)
only present in queens f 6a and f 7a
increased in queens f1, f3, f10, and f11
increased in workers f5 and f6 from Kitzingen (polygynous)
only present in queens f 7a
increased in queens f3, f5, f6, f7 and f11
increased in workers f0 and f8
increased in queens f1, f3, and f 7a from Rottenbauer (polygynous).
increased in workers f8 from Gramschatz (monogynous)
increased in queens f3, f6, f 6a and f10
increased in workers f11 and f14

Formica polyctena prepupae from Randersacker (polygynous)
present only in queens f2 and f5
present only in workers f4
increased in queens f3 and f11
increased in workers f0, f1, f7, f8 and f13 from Gramschatz (polygynous)
present only in queens f9, f 9a, f10 and f13
increased in queens f3, f4, f5, f8, f11a+b and f13
increased in workers f0 and f2

Without regard to the intraspecific differences, it could be demonstrated that prepupae of the queens have additional protein bands,

but these are mostly weakly stained in the pherograms. Some bands are more strongly coloured in the prepupae of queens than in those of workers. But no fraction could be found that was not evident during the metamorphosis of the two castes and sexes. Consequently the demonstrated differences in the electropherograms of the both castes suggest that a determination effect is present, in addition to the influence of differing organogenesis, which effects the lipid and carbohydrate metabolism (Schmidt 1967) and probably the protein synthesis (Rembold 1969 in the honeybee). The present relations and differences in the distribution of protein fractions indicate, furthermore, a different activation of genes, which have different effects in the single populations. This presumably results from a different secondary coupling of the reactions in metabolism, influenced by changing ecological factors. Different primary effects would seem to be improbable in such closely related species.



Scheme of a protein electropherogram of Formica in 7% polyacrylamid gels after staining with aniline black. The separated bands were numbered according to their running time; extention: 3 times.

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