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Further studies on the *Bracon hebetor-brevicornis*-complex (Hymenoptera: Braconidae) by paper chromatography

Introduction

There still exists a good deal of confusion on the identities of *Bracon brevicornis* WESMAEL and *Bracon hebetor* SAY and thus it is not possible to say with certainty whether these should be regarded as different species or that one is merely the variety or subspecies of the other. NARAYANAN et al. (1958) tried to clear the existing confusion on the basis of morphological characters (colour characters, number of antennal segments, male genitalia and wings) and biological studies. The latter included the experiments conducted to find out whether the two-forms interbred. However, the authors concluded "We are constrained to remark that either the original *hebetor* has undergone a large number of mutations and has started showing considerable variation in various morphological characters after having spread to many different climes since it was first described by THOMAS SAY or the species used by PUTTARUDRIAH and CHANNA BASAVANNA and ourselves is not the true *hebetor* but is a yet un-identified sibling species from amongst the *hebetor-brevicornis*-complex very near the true *hebetor*." With a view to clear this confusion the technique of paper chromatography as shown by MICKS (1956), was used to see if it can throw some light on this interesting and puzzling problem of *hebetor-brevicornis*-complex.

Material and Methods

Individuals of *Bracon* spp. i.e., *B. brevicornis*, *B. hebetor* and *B. gelechia* ASHMEAD were obtained from the cultures of these parasites maintained in the parasite laboratory of the Entomology Division, Indian Agricultural Research Institute, New Delhi. Parasites were starved for 6 hours and then 50 mgms. of each species were thoroughly rinsed with glass double distilled water to remove the extraneous matter that may be adhering to the surface of the parasite. Each lot was then ground with a little of anhydrous sodium sulphate and 95% ethanol. The homogenates, thus prepared, were centrifuged three times at 3,000 R.P.M. for 10 minutes every time. The supernatants were reduced to 0.2 ml. Later the descending uni-dimensional technique of paper chromatography of CONSDEN, GORDON and MARTIN (1944) was followed. There were three replications for each experiment and they were repeated 3 times. Sheets of WHATMAN filter No. 1

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were spotted with the material as well as the standards of the known amino acids. In each case 70 λ of concentrated volume of supernatants was spotted. This was determined by conducting preliminary experiments that the spots developed by 70 λ gave good results. The sheets were kept for drying for 12 hours after these were spotted. Later these were run three times in the solvent mixture consisting of n-butanal-glacial acetic acid and water (4:1:5 v/v/v) (SLOTTA and PRIMOSIGH, 1951). After each run the chromatograms were dried for 4–6 hours. After the last run, the chromatograms were sprayed with 0.1% ethanolic ninhydrin solution (BLOCK et al, 1955). These were then dried for an hour and then kept in the dark for 24 hours. Later spots were developed in 10 minutes in an electric oven at 85 to 90 °C. The spots of the unknown materials were identified by comparing the Rf values of the standards of known amino acids that were also run simultaneously on the same paper. A separate blank run side by side was also treated in the same manner as the spotted sheets to check up the possible impurities present in the paper.

Table

Spot No.	Standard	<i>Bracon brevicornis</i>	<i>Bracon hebetor</i>	<i>Bracon gelechiaie</i>
No. 1	Unidentified	+	+	+
No. 2	Serine	+	+	+
No. 3	Glycine	+	+	+
No. 4	Unidentified	+	+	+
No. 5	Hydroxyproline	+	+	+
No. 6	Histidine	+	—	—
No. 7	Valine	+	+	+
No. 8	Threonine	+	+++	+
No. 9	Norleucine	+	+	—
No. 10	Alanine	—	+	+
No. 11	Methione	+	+	+
No. 12	Proline	+	+	+
No. 13	Leucine group	+	+	+

Results and Discussion

As can be seen from the data presented in the Table — thirteen spots were developed on the paper. Out of these eleven could be identified as serine, glycine, hydroxyproline, histidine, valine, threonine, norleucine, alanine, methione, proline and leucine group. *Bracon brevicornis* contained twelve amino acids, i. e. ten identified as serine, glycine, hydroxyproline, histidine, valine, threonine, norleucine, methione, proline and leucine group and the two un-identified amino acids. Thus it did not contain alanine which was detected both in *Bracon hebetor* and *B. gelechiaie*. In *B. hebetor* though twelve amino acids were detected it differed from *B. brevicornis* in not containing histidine. Moreover *B. hebetor* contained about three times the quantity of threonine as compared to *B. brevicornis* and *B. gelechiaie*. The quantitative differences as judged from the intensity of the spot in this case were clear visually. However these differences were not well marked in other amino acids.

Bracon gelechia which is morphologically and otherwise recognised as distinct species contained only eleven amino acids. Histidine and norleucine could not be detected. Thus *B. gelechia* differed from *B. brevicornis* in not containing histidine and norleucine on the one hand while it was dissimilar from *B. hebetor* in not containing norleucine. *B. hebetor* contained alanine which was missing from *B. brevicornis*, thus it differed from it in this respect also.

Thus there do exist differences in the amino acid constituents in three *Bracon* forms studied and on the basis of these differences they can be considered as different from each other. However it would be worth while to undertake further quantitative studies of the amino acids constituents as well, and a study of chromosomes of these species. Such a study would throw more definite light and will help in understanding fully, the evolution of species of *hebetor-brevicornis*-complex.

Summary

The technique of paper chromatography has been used to clear the existing confusion on the identities of *Bracon brevicornis* WESMAEL and *Bracon hebetor* SAY. It has been shown that there do exist differences in the amino acid constituents in three *Bracon* forms studied. *B. brevicornis* contained twelve amino acids—serine, glycine, hydroxyproline, histidine, valine, threonine, norleucine, methionine, proline, leucine group and two un-identified amino acids whereas *B. hebetor* had serine, glycine, hydroxy proline, valine, threonine, norleucine, alanine, methionine, proline, leucine group and two unidentified spots. Only eleven amino acids could be detected in *Bracon gelechia*. These were serine, glycine, hydroxyproline, valine, threonine, alanine, methionine, leucine group and two unidentified spots.

Zusammenfassung

Das Verfahren der Papierchromatographie wurde benutzt, um die bestehende Unklarheit über die Identitäten von *Bracon brevicornis* WESMAEL und *Bracon hebetor* SAY zu beseitigen. Es wurde festgestellt, daß doch Unterschiede in den Aminosäurenkomponenten zwischen drei untersuchten *Bracon*-Formen bestehen. *B. brevicornis* enthielt zwölf Aminosäuren, nämlich Serin, Glyzin, Hydroxylprolin, Histidin, Valin, Threonin, Norleucin, Methionin, Prolin, die Leucingruppe und zwei nicht identifizierte Aminosäuren, während *B. hebetor* Serin, Glyzin, Hydroxylprolin, Valin, Threonin, Norleucin, Alanin, Methionin, Prolin, die Leucingruppe und zwei nicht identifizierte Punkte hatte. Bei *Bracon gelechia* konnten nur elf Aminosäuren festgestellt werden, nämlich Serin, Glyzin, Hydroxylprolin, Valin, Threonin, Alanin, Methionin, die Leucingruppe und zwei nicht identifizierte Punkte.

Резюме

Для устранения неясности об идентичности *Bracon brevicornis* WESMAEL и *Bracon hebetor* SAY был применен метод бумажной хроматографии. Установлено, что все-таки существуют различия компонентов аминокислот трех исследованных форм *Bracon*. *B. brevicornis* содержал 12 аминокислот, а именно: серин, глицин, гидроксилпролин, гистидин, валин, треонин, норлейцин, метион, пролин, группу лейцинов и две неидентифицированные аминокислоты, в то время как у *B. hebetor* были найдены серин, глицин, гидроксилпролин, валин, треонин, норлейцин, аланин, метион, пролин, группа лейцинов и две неидентифицированные точки. У *Bracon gelechia* было обнаружено только одиннадцать аминокислот — серин, глицин, гидроксилпролин, валин, треонин, аланин, метион, группа лейцинов и две неидентифицированные точки.

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Zeitschrift/Journal: [Beiträge zur Entomologie = Contributions to Entomology](#)

Jahr/Year: 1965

Band/Volume: [15](#)

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Artikel/Article: [Further studies on the Bracon hebetor-brevicornis-complex \(Hymenoptera: Braconidae\) by paper chromatography. 83-86](#)