The Genetic and Physiological Diversification of Two Species of Braconids, *Habrobracon juglandis* Ashmead and *Habrobracon serinopae* Ramkr.

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ABSTRACT

For the genetic and taxonomic diversity of two species of braconids, *Habrobracon juglandis* Ashmead and *H. serinipae* Ramkr., reciprocal cross; karyotype; pattern of protern and dehydrogenase isozymes of lactate, malate and xanthine; urate content during development were investigated. The results indicate that two of them should be different species.

In a total of sixty-five pairings of male *H. serinopae* and female *H. juglandis*, thirty-one females showed sperm in their spermathecae, but no sperm was found in female spermathecae in the crosses of male *H. julandis* and Female *H. serinopae*. No biparental offspring were obtained in both kinds of crossing.

Slight morprological difference between two species could not be giving as the taxonomic evidence. *H. juglandis* Ashmead and *H. hebetor* Say are the same species with different name, which is not the case for *H. serinopae* Ramkr.

The taxonomic ambiguity of braconid wasp species has contributed to confusion in the literature of genetics and in the use of these parasitoids for biological control of Lepidopteraan pests. Among the controversial issues is that *Habrobracon juglandis* Ashmead, known as *Bracon hebetor* Say to entomologists, is morphologically indistinguishable in dry museum specimens from *Habrobracon serinopae* Ramkr., first described by Cherian (1929) in India. On the other hand, fresh abdominal tissues differ in cellular details (Clark, 1967), and fecundity and life span differ markedly for the two types. To complicate matters, *H. serinopae* Ramkr. Has also been synonymized with *H. brevicornis* Wesmael (Rao *et al.*, 1948), but Lin (1965) compared various characteristics and concluded that the two forms were not identical. The present report summerizes evidence that *H. juglandis* and *H. serinopae* must be considered separate species although electrophoretic patterns for several enzymes were indistinguishable.

The *H. juglandis* used in this study was Whiting stock No. 33, from ancestors originally collected in California. *H. serinopae* stock was also obtained from P.W. and A.R. Whiting. When shipped from New Delhi, India to the United States the material was labelled as *H. brevicornis*.

In a total of sixty-five pairings of male *H. juglandis* and virgin female *H. serinopae* no biparental offspring were produced. Examination of spermathecae dissected from the females seven days after setting up the pairs revealed

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no sperm. Sixty-five reciprocal crosses were made at the same time. Although biparental offspring were not obtained, over 50% of the females showed sperm in their spermathecae. Evidently a reproductive block is well established.

Analysis of the karyotypes revealed no difference in the number of chromosomes in the two species. Ten pairs of chromosomes in the diploid females of each of the species were accurately counted. The morphology of the oogonial metaphase chromosomes of *H. juglandis* checked with those previously shown by Torvik-Greb (1935); four relatively large, three medium, two small and one dot-shaped pairs. These were, however, distinctly different from the *H. serinopae* chromosomes; four pairs appeared large, the others were medium, no very small rods (see Fig. 1).

The general protein patterns and the patterns of three enzymes, lactic dehydrogenase (LDH), malic dehydrogenase (MDH), and

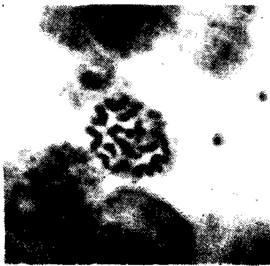
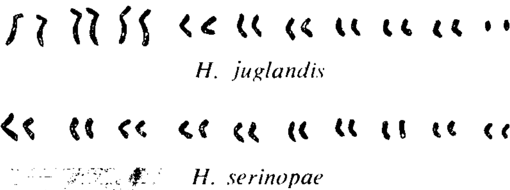


Fig. 1 A. Photomicrograph of the oogonial metaphase chromosomes in diploid *Habrobracon serinopae* female (×1125).



H. juglandis

H. serinopae

Fig. 1 B. Camera lucida drawings of the oogonial metaphase chromosomesin diploid *Habrobracon juglandis* ♀ and diploid *Habrobracon serinopae* ♀ (× 2000).

xanthine dehydrogenase (XDH) of the two species were studied by using polyacrylamide gel electrophoresis (Davis, 1964). Tissue extracts were prepared by homogenizing 300 mg of newly eclosed wasps into 6 ml of 0.1 M Tris buffer, pH 8.0, containing 1 M sucrose at 0°C. The homogenate was thoroughly mixed with 600 mg norite-A and allowed to stand for 20 min. in an ice bath to remove endogenous inhibitors of the enzymes. At the end of this period the slurry was centrifuged twice at 30,000×g, each for 20 min.. The fatty layer which floated on the top was removed by passing the supernatant through a piece of glass wool.

Electrophoresis was performed in columns each of 1.3 cm in diameter and 7.5 cm in length. 0.1 ml of the sample was place directly on the top of the gel. A direct current of 4 mA per gel column was applied for a period of 7 hours. The temperature was maintained at 20°C. The general protein patterns were made visible by staining the gel in 0.5 per cent amido black 10B in 7 per cent acetic acid followed by electric destaining. The zymograms of the three dehydrogenases were made by incubating the gel blocks in the reagent mixtures at 30°C in the dark after electrophoretic separations were complete. 1×10^{-6} M DL-lactic acid, 3.6×10^{-2} M DL-malic acid and 3×10^{-5} M hypoxanthine were used as substrates for LDH, MDH, and XDH respectively. Other components of the reagent mixtures were similar for all three enzymes: 0.3 mg/ml nicotinamide adenine dinucleotide (NAD); 0.14 mg/ml phenazine methosulfate; 0.3 mg/ml nitro-blue tetrazolium; in 0.1 M Tris buffer, pH 8.0-8.3. Controls in which the substrates were deleted were set up for determining the specific enzymes under study.

In both species there were 11 protein bands with very similar mobilities. The enzyme

patterns were also similar in the two species for all the three enxymes studied. Each gel block incubated in the complete system of any of the incubation mixtures showed two bands. The one near the origin was not specific to the substrate added, since it also showed up in the control blocks. The mobilities of the specific enzyme bands indicated no differentiation of the enzyme structures in the two species (Fig. 2).

A study of the quantitative difference of XDH activity of the two species was carried out because of the apparent difference of urate cell size in the two species (Clark, 1967). The enzyme was assayed by determining spectrophotometrically the increase of uric acid when hypoxanthine was used as substrate (Lin, 1965). Haploid males and diploid females were determined separately. Unexpectedly there were no differences between the corresponding sex types of the two species. Within each species the diploid females had higher XDH activity than the haploid impaternal males.

Measurement of the uric acid content in the body of various developing forms and adults of different ages by means of an enzymatic method (Kalckar, 1947) showed uric acid accumulation in *H. juglandis* consistantly higher than *H. serinopas* except on day 16. At this time the contents in all types were almost the same (Fig. 3). The difference in amount of urate, however, may well be an indication of the physiological diversification of the two species. Transfer of substances from urate cells to the ovarioles for the construction of egg materials has been shown by using C-14 labelled hypoxanthine. The results will be the topic of another paper present elsewhere. Significantly higher fecundity of *H. serinopae* females may have direct correlation with its lower uric acid content. Since the qualitative aspects of

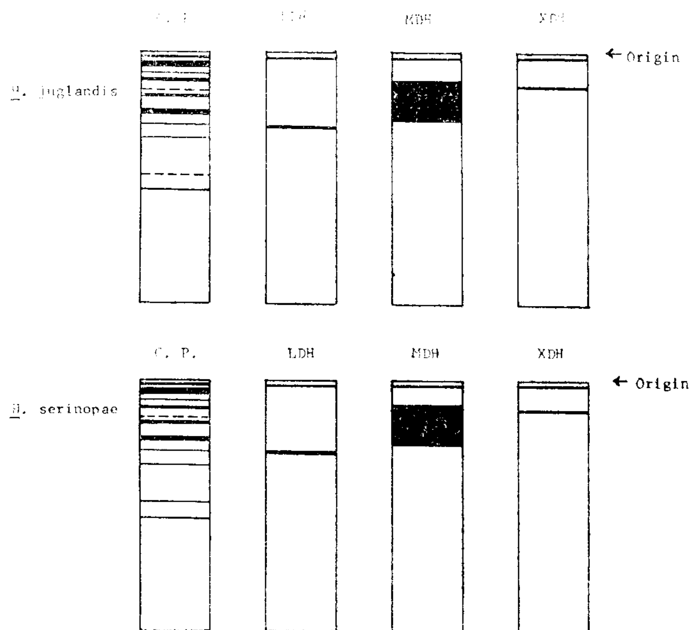


Fig. 2. Diagramatic drawings of the general protein patterns and the zymograms of three enzymes, LDH, MDH, and XDH in the two species. G. P.-general protein pattern. Solid lines indicate dark staining bands. Dotted lines indicate faint bands. LDH, MDH, and XDH-zymograms of the three enzymes: lactic, malic, and xanthine dehydrogenases. The band near the origin in each of the zymogram is not specific to the substrate added.

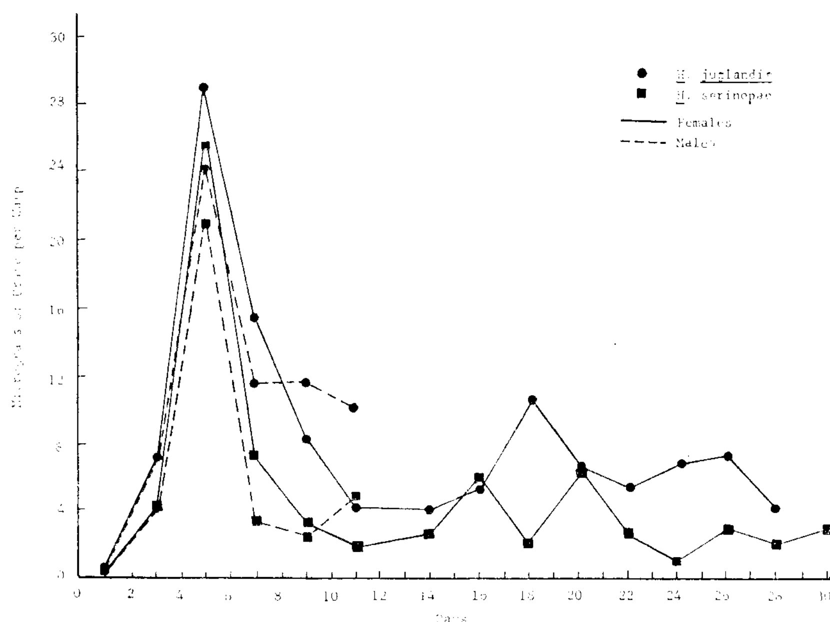


Fig. 3. Urate content of the two species during development and after eclosion. *H. serinopae* ecloses at the 9th day after the egg is laid, and *H. juglandis* at the 10th day. *Hjuglandis H. serinopae.*

the XDH showed no distinction in these insects, the physiological divergence may be in the metabolic steps before the formation of the hydroxypurines and/or, most likely, in the utilization of the purine derivatives stored in the urate cells.

To date, physiological diversification has been demonstrated only for the storage-excretion pathway. Reproductive isolation and karyotypic differences were also reported above. Possibly selective forces acting on physiological adaptability are more intensive than on morphological features. In any event, present findings point up the importance of not confining attempts at classification to strictly macro-morphological characters.

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小繭蜂 *Habrobracon juglandis* Ashmead 及 *Habrobracon serinopae* Ramkr. 遺傳及生理上的趨異

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摘　　要

小繭蜂 ***Habrobracon juglandis*** Ashmead 及 ***H. serinopae*** Ramkr. 兩種在遺傳學及分類學上的混淆，經單對配交；染色體比較；一般蛋白質、乳酸去氫酶、蘋菓酸去氫酶及黃嘌呤去氫酶之電泳譜，及尿酸儲積量在發生過程中之不同，斷定其為兩個完全分離之生物品種。在 65 對 ***H. juglandis*** ♀ ×***H. serinopae*** ♂ 配交中 31 隻雌蜂儲精囊內有精子，65 對 *H. serinopae* ♀ ×*H. juglandis* ♂ 配交中所有雌蜂儲精囊內均無精子，而所有配交均未產生雜種子代。兩種在形態上些許的歧異不足以用為分類學的依據，***H. juglandis*** Ashmead 及 ***H. hebetor*** **Say** 為同種異名，而與 *H. serinopae* Ramkr. 不同。