

Breeding experiments with Biological races of *Trichogramma minutum* in the West Indies

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Introduction

The hymenopterous egg parasite, *Trichogramma minutum* (RILEY), is being used in various parts of the world for the control of certain lepidopterous pests such as for example the sugar-cane moth borer, (*Diatraea* spp.), and the Oriental fruit moth *Cydia (Laspeyresia) molesta*, BUSCK. It appears from our somewhat meagre knowledge of the biology of this species that it exists in an undetermined number of races differing in morphological characters such as colour, and in such physiological characters as host preference, length of life history, reaction of colour to temperature etc.

The type of parthenogenesis—whether virgin females are male or female producing, also provides an important biological difference

existing in the species. The occurrence of wide physiological differences in association with identity of morphology has provided the taxonomist with a problem which is involved in some confusion, namely, how many species of *Trichogramma* really exist. The solution to this problem can only be provided as the outcome of an extensive study of the biological characteristics, crossing relationships, cytology, and genetics of the various races. It is felt that such a study should precede, or at any rate accompany the attempts now in progress to domesticate and employ this insect for economic purposes. Much knowledge of methods for the mass production of *Trichogramma* has been accumulated without realising that the *Trichogrammas* liberated probably represent a huge reservoir of genetic variability.

The senior author has previously published (1915) the results of observations on hosts of *Trichogramma* in the West Indies, and the present study was begun in 1930 as a result of a statement made by R. W. E. TUCKER (personal communication) to the effect that in Barbados certain strains of *Trichogramma* did not take readily to the laboratory host *Sitotroga cerealella*. From the genetics point of view the differences shown by insects in their preference for certain food plants, or the differences exhibited by various races of a parasite towards a series of hosts, are of extreme interest. Since *Trichogramma minutum* occurs in every island of the West Indies on a large number of hosts, it seemed to us probable that each island might contain one or more races exhibiting analysable physiological differences. The object of the experiments described in this paper were therefore to collect strains of *Trichogramma* from various localities in the West Indies, to examine their morphological and physiological characters, and by means of controlled crossing experiments to obtain information on their relationships.

Previous investigations

No attempt will be made to deal with the large number of sporadic allusions to *T. minutum* which occur scattered through the literature. Many of the references have not been available, and of the rest a considerable number have been seen only in abstract form. It is hoped, however, that the most important papers on the biology and breeding of *Trichogramma* have not been overlooked.

HOWARD and FISKE (cited by FERRIERE and FAURE 1924) working in U.S.A. received from Europe *Trichogrammas* which they separated

into two species, one which was morphologically identical with the American *T. minutum* but differed in producing either both sexes or females only by parthenogenesis, the offspring of unfertilised females belonging to the American species being invariably composed of males. The other European species was recognisable by its dark colour. Like the American species, males only were produced by parthenogenesis. FERRIÈRE and FAURE consider that in the two races, morphologically identical but biologically distinct, of *Trichogramma*, (American and European) there are really two species, as distinct as the species of bacteria established on cultural differences.

HASE (1925) states with reference to *T. evanescens* WESTW., that when eight different host eggs were presented, no preference for any one was shown, and parasites bred from one species readily attacked the eggs of all others. As will appear from the observations described in this paper, this is not true of the West Indian races of *T. minutum*.

MARCHAL (1927) studied two closely related forms of *Trichogramma*. He described the colour differences between these, which he designates as *T. evanescens*, (typical form), and *T. cacaeciae* (sp. n.). The former is found chiefly in cabbage beds, where it parasitises the eggs of *Pieris brassicae* and other insects, while the latter is found in shady places on apple and old quince trees where it parasitises the eggs of *Tortrix (Cacoecia) rosana* L., *T. evanescens* produces males by parthenogenesis while *T. cacaeciae* produces usually females, males appearing only very exceptionally. The eggs of *Tortrix rosana* were not parasitised by *T. evanescens*, however favourable the conditions. Attempts to cross the two species were unsuccessful.

FLANDERS (April 1930) states that strains of *T. minutum* from different localities may exhibit marked differences in fecundity when reared under the same conditions. He essayed crosses between the California strain (yellow) and the Mexican strain (dark) but although mating occurred the progeny was all male, indicating non-sexual reproduction.

FLANDERS (July 1930) studied strains of *T. minutum* from Mexico and various localities in the United States and established five different races on the basis of colour and length of life cycle.

PETERSON (1930) working in New Jersey established the existence of two distinct forms of *T. minutum*. Both were morphologically indistinguishable, but during the summer the females of one type are distinctly lemon-yellow in colour, while the females of the other are

olivaceous—brown particularly the abdomen and the thorax. Extensive crossing experiments were tried but the females produced only males, showing that crossing did not take place.

The same author (1931) found that females of a yellow race of *T. minutum* were able to withstand longer and more severe refrigeration than females of the dark race when reared in bag-worm eggs. Females of the yellow race lived twice as long as those of the dark race, and were four times as prolific when reared in eggs of the bag-worm. Attempts to cross a race with yellow females by a race with darker and smaller females were unsuccessful. The progeny from such crosses consisted solely of males.

The experimental Results

Methods

As hosts for laboratory culture of the various races of *T. minutum* two species of moth attacking stored maize have been used, viz. *Sitotroga cerealella*, OLIVIER and *Corcyra cephalonica*, STANTON. The former has the advantage that the larvae do not eat the parasitised eggs, but as will follow, it is not susceptible to parasitism by all our strains. *Corcyra* is an excellent host and the habit of the newly hatched larvae of eating the parasitised eggs can be largely minimised by putting a raisin in the culture tube before hatching is expected.

Material

The races have been classified into three groups on the basis of colour differences and on the type of parthenogenesis involved. In addition to nine West Indian strains, two American strains were used for comparison.

The existence of races of *T. minutum* producing only females by parthenogenesis has not been previously recorded from the New World.

(1) Yellow group producing males by parthenogenesis	Grades of Yellow
American yellow short cycle (C)	} 1
American yellow transition (B)	
Trinidad yellow (DD)	
Antigua (AN)	3
Barbados local (A)	} 4
Trinidad (DA)	
(2) Yellow group producing females by parthenogenesis	
St Lucia (SV)	} 2
St Vincent (SL)	

The colour of these two strains falls in between grades 1 and 3, from which it may clearly be distinguished.

(3) Dark group producing males by parthenogenesis

Trinidad	(DB) and (DC)
Montserrat	(M)
St Vincent	(V)

Further notes on Colour. (1) Yellow group

Grade 1

In the yellow group, the American strain B and C, and the Trinidad DD strain are approximately equal in degree of pigmentation. There is possibly an imperceptible bias towards a stronger yellow in the DD strain, but although a comparison of mass cultures would reveal the difference, it is not possible to separate out individuals on this basis. All three strains are therefore classified as grade 1.

Grade 2

SL, St Lucia, and SV, St Vincent are the next strains in order of yellow colour. This grade is characterised by a varying amount of dark pigmentation on the abdominal segments, such pigmentation being lacking in grade 1. The pigmentation is most intense at the junction of one segment with another, and diminishes on either side.

Grade 3

In this grade the pigmentation of the abdominal segments becomes more intense in the inter-segmental regions, imparting to the abdomen a distinctly darker appearance which can be seen even without the aid of a lens. The yellow pigment of the thorax is darkened. It is possible to distinguish this grade from 1 and 2, in single individuals, there being no overlapping.

Grade 4

The characteristic yellow of the thorax disappears, being replaced by a weak development of the dark pigment. The colour of the thorax is best described as a dilute smoky black.

(2) Dark group

In this group the colour is much darker than the darkest of the yellow group. All three strains (Trinidad DB, Montserrat M, and St Vincent B) are similar in intensity of pigmentation.

Origin and host preference of strains

The strains are dealt with in the order in which they have been classified in the previous section.

B. — *American yellow transition*

Of Californian origin. Parasitises *Sitotroga* and *Corcyra*. Attracted to eggs of *Calpodes ethlius* CRAM., but apparently not able to breed on this host.

C. — *American yellow short cycle*

Origin — Massachusetts. Parasitises *Sitotroga* and *Corcyra*. Reaction to *Calpodes* similar to B.

DD — *Trinidad yellow*

Obtained from a single cluster of eggs of *Diatraea* sp. on maize. *Sitotroga* and *Corcyra* eggs immediately parasitised. Vigorous attempts made to oviposit in *Calpodes* eggs but no parasites hatched.

AN — *Antigua*

A large mass culture from *Diatraea* was received from Antigua and the emerging parasites tested with eggs of *Sitotroga*, *Corcyra* and *Calpodes*. Eggs of *Sitotroga* were completely ignored while eggs of *Corcyra* were also taken no notice of during the period of observation. A few eggs of *Corcyra* were however ultimately parasitised and the parasites from these exhibited strong attraction towards *Corcyra* in the next generation and the Antigua strain has been continued up to the present on this host. No attraction towards *Sitotroga* has ever been exhibited at any time.

Two *Calpodes* eggs placed in contact with the original mass culture gave rise to both males and females. The females refused to parasitise either *Sitotroga* or *Corcyra*. The *Calpodes* eggs were obtained from the field and it is not certain that previous parasitism by a Trinidad strain had not taken place.

The fact however that all Trinidad strains from *Calpodes* have parasitised *Corcyra* successfully seems to indicate that these parasites were of the Antigua strain and that *Trichogrammas* from *Diatraea* in Antigua were genetically diverse, at least two types existing, one positive and the other negative to *Corcyra*.

A — Barbados local

Bred from *Diatraea* by R. W. E. TUCKER and used by him in Barbados in his experiments on the mass breeding of *Trichogramma*. Parasitises both *Sitotroga* and *Corcyra*. Reaction to *Calpodes* not known.

DA — Trinidad

Originated from *Sitotroga* eggs parasitised by *Trichogramma* received from Mr. F. W. URICH who stated that they were from *Calpodes*. This strain parasitises both *Sitotroga* and *Corcyra* but its reaction to *Calpodes* has not been tested owing to the difficulty of obtaining *Calpodes* eggs unaffected by parasites in the field.

SL — St Lucia yellow

Collected in St Lucia by one of us (S. C. H.) from *Diatraea* eggs. It was tested by R. W. E. TUCKER in Barbados and took readily to *Sitotroga* eggs. In Trinidad it has been propagated indiscriminately on both *Sitotroga* and *Corcyra*. Reaction to *Calpodes* is not known.

SV — St Vincent yellow

Obtained by F. W. URICH from eggs of *Chloridea virescens* (?) on tomato.

DB and DC — Trinidad

The strain DB was hatched out of *Sitotroga* eggs parasitised by females received from Mr. URICH. The interesting fact emerged that although hatching from *Sitotroga*, the strain refused to parasitise this host in the next generation, and has since failed to do so. It has therefore been propagated on *Corcyra*.

It is clear that even when the parasite is not normally attracted towards a given host, parasitism may occur accidentally in a small proportion of cases. If such parasitism is merely accidental the next generation should exhibit an indifference towards the host similar to that of the majority of the preceding generation, as was here noted.

This case is to be distinguished from the Antigua one described above, where although *Corcyra* was initially only lightly parasitised, the descendants from the parasitism were capable of complete parasitism in the next and succeeding generations.

The strain DC obtained from *Calpodes* eggs from Canna near the laboratory may be identical with DB.

M — Montserrat

Obtained by the senior author from *Calpodes* eggs. The parasite refused to oviposit on *Sitotroga*, and as *Corcyra* was not available at that time as a host, the strain could not be carried on.

V — St Vincent dark

This strain was obtained from St Vincent from *Diatraea*. Similar to the Montserrat strain in all respects, and has thus also been lost.

Before continuing further, the most important fact so far presented may be briefly summarised in the following statement.

The West Indian strains of *T. minutum* may be divided into at least seven races, distinguishable on the basis of colour, type of parthenogenesis, and host reaction.

Types of Parthenogenesis

The existence of a race of *T. minutum* in Europe producing females by parthenogenesis has been frequently referred to in the literature. No such race however has previously been recorded from any part of the New World. The St Lucia race was discovered in the course of the crossing experiments about to be described and it was found that if St Lucia females were confined with males belonging to any other race the progeny consisted solely of females identical in all respects with the St Lucia form, one solitary male appeared in a single culture. Subsequently, it was noticed that after two or three generations of mass culture the males completely died out and the strain has since been carried on parthenogenetically through the females. The St Vincent race has proved to resemble closely that from St Lucia, although until crossing experiments can be carried out the identity of the two must be left in doubt. It should finally be stated that the original mass culture from St Lucia must have contained a mixture of male and female producing strains, for our records shew that one of the original virgin females gave rise to males only.

Crossing Experiments

Many crossing experiments between the various strains have been attempted. All combinations could not be made, since some of the

strains were lost through want of a suitable host, and others died out through weakness. Nevertheless, the number of combinations made is sufficiently extensive to draw certain conclusions.

Crosses between members of the yellow or light group

1. B and C

In this experiment seven virgin females of the B race were used. Each was put into a small glass tube about an inch in length and allowed about $\frac{1}{4}$ inch space in which to move about. A C male was introduced and when copulation took place the pair were transferred to a larger tube containing a fresh card of host eggs. On the eighth day 25 parasites emerged comprising both males and females. This cross so readily made has since been carried on for many generations, but no observations have been made on the variability of the length of the life history, since this was outside the main scope of the work. It is evident that these two American forms are merely physiological subtypes of the same species.

2. B (American transition long cycle) and DD (Trinidad)

Single virgin females of strain DD were confined, each with a single male. To test the adequacy of the method a control experiment in which a DD ♀ was confined in a small tube with a DD ♂ was carried out. In the control experiment mating was observed to take place almost immediately, and the female made no attempt to escape. From this mating nine offspring emerged, six females and three males. This method of mating has been used throughout these experiments.

The results of crossing experiments between DD ♀s and B ♂s are placed below in Table 1 (see pag. 63).

From the above results it will be seen that although the B ♂s are sometimes attracted to the DD ♀s, and that mating apparently occurs, the union is unfruitful, and in seven cases out of nine, the female died without ovipositing. It seems likely that mating may be the cause of early death, since the control female lived for several days. In the two experiments in which offspring were obtained, only parthenogenetic males of the DD strain were obtained. It is thus probable that DD ♀ and B ♂ will not intercross. It may be remarked that the DD strain is rather weak in captivity and has since died out.

Table 1. DD♀ × B♂ (single individuals)

Expt.	Observations on mating	Host	No. of eggs parasitised	No. parasites emerged		Remarks
				♀	♂	
41-1	♂ attracted; tried to copulate with ♀ on two occasions in 5 minutes; ♀ tried to escape. Mating not effected	Corcyra	22 out of 50	0	10	Like DD strain Cross unseccessful
41-3	Mating not effected. No attraction	do.	28 ,, 50	0	22	do.
41-4	Mating observed after 10 minutes. Attraction very slight	do.	0 ,, 50	0	0	♀ died without ovipositing
41-5	No mating and no attempt by ♂ in 10 minutes observation	do.	—	—	—	♀ died
41-6	Male not attracted after 15 minutes	do.	0 out of 50	0	0	—
41-7	Male attracted. Mating almost immediately	—	—	—	—	♀ found dead next morning
41-8	do.	—	—	—	—	do.
41-9	Male attracted. Mating after 3 minutes	—	—	—	—	do.
41-10	Male attracted. Mating after 8 minutes	—	—	—	—	do.

3. B (American yellow transition) and AN (Antigua)

Only one cross was attempted, with the following results.

Table 2. B♀ × AN♂

Expt.	Observation on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♂	♀	
20-1	No attraction; mating not observed	Sitotroga	11 out of 36	0	7	Identical with ♂s of B strain

From the fact that there was not the slightest sexual attraction between the pair, it may be concluded that this cross is difficult, if not impossible to effect.

4. B (American yellow transition) and A (Barbados local)

A large number of experiments have been made, using both types as female and male. In only one case was mating attempted, and this occurred when two B♀s were confined in a small space with a number of A♂s. This cross has been tried to a far greater extent than any of the others, and it is certain that this cross is not possible to make. The almost complete absence of any sexual attraction is the most significant fact in this connection.

5. B (American yellow transition) × DA (Trinidad)

Two experiments were made. The results are presented in Table 3.

Table 3. B♀ × AN♂

Expt.	Observation on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	♂	
10-1	No attraction; mating not observed	Coreyra	28 out of 40	0	27	Identical with B B♂s strain
10-2	do.	do.	8 „ 40	0	all	do.

These two crosses gave negative results.

6. DD (Trinidad) × A (Barbados local)

The results of attempts to make this cross are detailed below in Table 4.

Table 4. DD♀ × A♂

Expt.	Observation on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	♂	
40-1	No attraction; ♂ paid no attention to ♀	—	—	—	—	♀ died without laying
40-2	do.	—	—	—	—	3♀s confined with 3♂s. Females died without laying
40-3	do.	Coreyra	12 out of 50	0	12	Like DD♂s

The complete absence of sexual attraction is again to be noted.

7. DD (Trinidad) \times DA (Trinidad)

The results of three crosses are placed below in Table 5.

Table 5. DD♀ \times DA♂

Expt.	Observations on mating	Host	No. of eggs parasitised	No of parasites		Remarks
				♀	♂	
42-1	No attraction. ♂ paid no attention to ♀	Corcyra	4 out of 50	0	6	Like DD ♂s
42-2	do.	do.	11 „ 50	0	12	do.
42-3	do.	do.	9 „ 50	0	5	do.

Here it is remarkable that two yellow types, both from Trinidad differing only slightly but consistently in colour, should not only be uncrossable, but should show no sexual attraction whatever.

8. A (Barbados local) and AN (Antigua)

The results of three crosses are placed below in Table 6.

Table 6. A♀ \times AN♂

Expt.	Observations on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	♂	
18-1	No attraction; no mating	Corcyra	18 out of 90	0	6	All ♂s identical with A race. Some parasitised eggs eaten and parasites did not emerge
18-2	Mating observed as soon as ♀ introduced	do.	23 „ 50	8	8	Cross successful. All ♂s died next day although fed
18-3	Attraction slight. Mating not observed	do.	18 „ 50	0	15	All ♂s identical with A race

The main points of importance are as follows:

(1) Sex attraction varied from pair to pair, and in one case (Expt. 18-1) there appeared to be no attraction whatever. The cause of such variability is not known, but is possibly to be traced to differences in genes influencing crossability.

(2) In Expt. 18-2 a successful cross was obtained. The early death of the ♂s is somewhat remarkable, since ♂s of the two parent types when fed live usually for several days.

Subsequent Generations

The eight F₁-females which were fertilised by males which were presumably haploid and therefore genetically A, were isolated and bred from.

In regard to colour, five were slightly lighter than AN, and three were indistinguishable from AN.

No. 1. This female was a trifle lighter in colour than the AN♀, though not so yellow as the St Lucia female. It produced 23 offspring from *Corecra*, of which 17 were females and 6 males. The 6 males would presumably be haploid and possess all the genic combinations of the two parent types. Crossing was allowed to take place en masse and a family of 100 was raised, using *Sitotroga* as a host. It has previously been noted that AN refuses to parasitise *Sitotroga* at all, and the fact that some at least of the hybrids were able to employ this host, is of distinct importance. Two types of females could be recognised, a more abundant light form like the AN type, and a darker form closely resembling the Barbados type. Some variation in colour was also exhibited by the males. It is not implied that segregation of colour was simple, since there occurred a series of colour forms imperceptibly grading from one parent to the other. This female was the only one which proved fertile.

Notes were made on the other F₁-females as follows:

No. 2. Very weak and showed no attraction to eggs of either *Corecra* or *Sitotroga*. Died the next day after emergence. Colour like AN♀.

Nos. 3, 4, and 5. No attraction towards eggs of *Corecra*. Died when four days old without ovipositing. Somewhat lighter than AN in colour.

Nos. 6, 7, and 8. Behaviour identical with Nos. 3, 4, and 5, but colour indistinguishable from AN. Died in four days.

Thus of the eight females, seven were weak and functionally sterile, and only one produced offspring. This fact shows that considerable incompatibility exists between the Barbados and Antigua races, even though hybridisation can be effected.

The hybrid strain has been mass cultured on *Sitotroga* from 29th January to 20th June, with one new generation approximately

every 8—9 days. Examination of the culture at the end of this period showed that variability in colour had practically disappeared and the females closely resembled the Antigua type, the dark Barbados type having been eliminated. We therefore have a new race developed through crossing, with the colour characteristics of the Antigua race, combined with the ability to parasitise *Sitotroga* of the Barbados race.

It is our intention in future to make further crosses between these two races in order to elucidate the mode of inheritance of ability to parasitise *Sitotroga* eggs. The results of such an experiment should be of great interest from an evolutionary standpoint.

Crossability within the dark group

1. M (Montserrat) \times V (St Vincent dark)

Opportunity was afforded to try one experiment only. Three M♀s were confined with three V♂s. No attempt was made to mate, and since the females refused to parasitise *Sitotroga*, and no other host was at that time available, the experiment could not be further continued.

So far as they go, however, the results fall into line with those previously discussed, namely, that races from the different islands of the Lesser Antilles do not as a rule intercross.

Crossability of the light and dark group

1. DA (Trinidad) and DB (Trinidad)

$$DB\text{♀} \times DA\text{♂}$$

One experiment only was carried out. The pair showed no signs of willingness to mate during the 30 minutes observation. No parasitised eggs were obtained.

2. DC (Trinidad) and A (Barbados)

$$\begin{array}{cc} \text{Dark} & \text{light} \\ DC\text{♀} & \times A\text{♂} \end{array}$$

One experiment was tried. The male was attracted, and mating was attempted. The female died without ovipositing.

Both these experiments are inconclusive, and further experiments on a larger scale will be carried out.

Crossability of the light group (δ producing) and the light (♀ producing)

1. SL (St Lucia) and B (American Yellow short cycle)

As in previous experiments, single pairs were confined together. The results are presented in Table 7, below:

Table 7. SL ♀ \times B δ

Expt.	Observations on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	δ	
2-1	No attraction; after being together 15 minutes, no attempt at mating	Corcyra	—	—		♀ died without laying
2-2	No mating observed after being together 10 minutes	do.	6 out of 50	6	0	All ♀ s like ♀ parent
2-3	do.	do.	57 „ 80	many	0	do.

These three experiments indicate complete incompatibility between SL and B.

2. SL (St Lucia) and AN (Antigua)

Two experiments were made. The results follow in Table 8.

Table 8. SL ♀ \times AN δ

Expt.	Observations on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	δ	
36-1	Some attraction; mating observed after 10 minutes	Corcyra	18 out of 50	18	0	All like SL ♀ s
36-2	No attraction; no mating	—	—	—	—	♀ died before ovipositing

Cross unsuccessful.

3. SL (St Lucia) and DD (Trinidad)

This experiment was carried out successfully and in some detail. The results are set out in Table 9, below (see pag. 69).

Here offspring were produced in three cultures out of nine, but they were all female and identical in appearance with SL ♀ s. In some cases the females were allowed to oviposit, but continued to give only the SL ♀ -type. The cross was therefore unsuccessful.

Table 9. SL ♀ × DD ♂

Expt.	Observations on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	♂	
34-1	No attraction. No mating observed	Corcyra	15 out of 50	15	0	Identical with SL ♀s
34-3	do.	do.	1 „ 50	—	—	—
34-4	Male attracted. Mating observed immediately	do.	19 „ 50	—	—	Results not recorded
34-5	Male slightly attracted. Mating attempted	—	—	—	—	♀ died before eggs could be given
35-6	As 34-4	Corcyra	12 out of 50	11	0	Identical with SL ♀s
34-7	As 34-5	—	—	—	—	♀ died without laying
34-8	As 34-4	—	—	—	—	do.
34-9	As 34-4	—	—	—	—	do.
34-13	As 34-4	Corcyra	2 out of 150	2	0	SL in appearance

4. SL (St Lucia) and A (Barbados)

Three experiments were made. The results are placed in Table 10.

Table 10. SL ♀ × A ♂

Expt.	Observation on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	♂	
1-1	Mating took place about 20 minutes after introduction of males	Sitotroga and Corcyra	none	none	none	♀ did not lay. Reason unknown
1-2	No attraction. No mating observed	Corcyra	13 out of 39	9	0	♀ removed after 2 hours. All ♀s were of SL type. Strain continued parthenogenetic to give nothing but SL-type
1-3	Attempted mating. Some attraction	Sitotroga and Corcyra	3 out of 20	3	0	All of SL type

Females from Expts. 1-2 and 1-3 were again mated with A♂s but gave rise to females only identical with SL♀s. It may therefore be concluded that the cross between SL♀ and A♂ cannot be effected. No crosses could be made using SL♂s as they were lost.

5. SV (St Vincent) × B (American)

Four crosses were attempted. The results follow in Table 11.

Table 11. SV♀ × B♂

Expt.	Observations on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	♂	
51-1	Mated almost immediately male was introduced	Sitotroga	9 out of 100	3	0	SV-type
51-2	Immediate mating	do.	15 ,, 50	6	0	SV-type
51-3	do.	do.	18 ,, 50	1	0	SV-type
51-4	do.	do.	15 ,, 50	6	0	SV-type

Parasitism in these experiments is not high. All the females produced are evidently by parthenogenesis, since they are of the St Vincent type and show no trace whatever of the B parent. It may be assumed that SV and B do not cross, and that the SV-type is closely allied to, though it may not cross with the SL-type which produces parthenogenetic females.

Crossability of the light (♀ producing) and the dark group

1. SL (St Lucia) and DB (Trinidad)

Two crosses were attempted, the results of which follow in Table 12.

Table 12. SL♀ × DB♂

Expt.	Observations on mating	Host	No. of eggs parasitised	No. of parasites		Remarks
				♀	♂	
4-1	No attraction and no mating	Coreyra	14 out of 100	12	0	Identical with SL♀s
4-2	do.	do.	9 ,, 50	9	0	do.

The complete lack of sexual attraction in both experiments leads to the belief that crossing between these forms is not possible.

Discussion

Since crossing for the most part is absent between the races of *Trichogramma* described in this paper, and since several races may co-exist in a given area and upon a given host, it is clear that they must be considered from an economic point of view just as if they were separate species. From the economic point of view it is necessary to assess the value of each of the separate races in terms of their suitability for the biological control of any lepidopterous pest.

The facts presented in this paper provide further illustration of the profound differences in physiology which may exist in biological races of an organism unaccompanied by corresponding morphological differences. Little is known of the genetics of physiological characters but there is no reason to suppose that they are not subject to the same laws as those governing the inheritance of morphological characters. The fact that a new race has been synthesised by hybridisation with the colour characteristics of one race (Antigua) and the host preference of another (Barbados), is a strong indication that genetic factors for host reaction are involved, and this preliminary study opens up the way to a detailed investigation of the mode of inheritance of ability or willingness to parasitise *Sitotroga*.

The question why so little morphological variation is apparent in these biological races of *Trichogramma* may be briefly discussed. It is probable that in an insect such as *Trichogramma*, the morphological and anatomical adjustment to the environment may have to be so precise that there is little or no scope for variability. It is also likely that the supposed morphological identity of different races may be illusory and merely the result of insufficiently detailed examination.

STURTEVANT (1919) showed that *Drosophila simulans*, STURT., could only be separated from *Drosophila melanogaster*, MEIGEN., by one satisfactory character viz. the external male genitalia. STURTEVANT states: "Though experience will give one the impression that the two forms are distinct and should be classed as "good species", they are so close that a technical description of *D. melanogaster* or a drawing of the style familiar to geneticists would pass equally well for *D. simulans*. So far as I have been able to discover, the mating and breeding habits and the length of the life cycle are identical. Dr. C. W. METZ reports after a preliminary study that the chromosome groups are probably also identical. I am unable to distinguish the larvae, the pupae, or

the female genitalia. The structure of the male genitalia and the sterility of the hybrids remain as the only valid grounds for making a specific distinction; but I think there can be no question that those grounds are sufficient".

LANCEFIELD (1929) studied crosses between two physiological species (biological races) of *D. obscura*, FALLEN. He found that stocks of *D. obscura* were of two types identical in appearance but differing in genetic behaviour. Crosses between the two types were made with some difficulty, resulting in sterile sons and partially fertile daughters. The only morphological difference found was in the size and shape of the Y-chromosome. LANCEFIELD observes that the two races of *D. obscura* are less differentiated from each other than are *D. melanogaster* and *D. simulans*, and may represent an earlier step in the evolutionary process.

PLOUGH (1924) has published a case of a self-fertile strain of *D. melanogaster* which is partially sterile in out-crosses. Information was only given in abstract form, but as LANCEFIELD observes, "It would seem that such an initial block to free interbreeding might well serve as a starting point for differentiation, and the accumulation of many genic differences to any mutual incompatibility of the chromosomes from the two sources".

The three cases in *Drosophila* referred to above seem to constitute stages of the evolutionary process and to indicate the impossibility of defining exactly the criteria of a species in terms of morphological or physiological characters. This is mainly due to the fact that the variation in physiological and morphological characters and in the factors determining crossibility do not follow, *pari passu*, variations in biochemical composition referable ultimately to the numbers and kinds of genes involved.

The only true criterion of the degree of relationship between two organisms is, in the last analysis, biochemical, involving specificity of organic compounds. This point of view is illustrated by the results of modern studies of sero-diagnostic relationships. It is improbable that the organic world can be divided up into completely discontinuous entities termed species, since the possession of a common gene such as that for albinism in Mammalia implies biochemical identity in respect of the chemical reactions initiated by such a gene. It is probable that the whole of the Mammalia or Insecta possess many genes in common so that we should expect to find evidences of biochemical continuity in these groups. Sero-diagnostic tests provide such evidence.

The taxonomist holds that the test of a species lies primarily in its external morphology and this has frequently led him to describe a Mendelian variation dependent on a single gene as a separate species. From the genetic point of view, a genic combination leading to the production of a morphological character is no more important than another genic combination leading to the production of a physiological character. For anything we know to the contrary and since the two are known to be crossable, there might be more genic resemblance between say a pheasant and a domestic fowl than between some of the races of *Trichogramma* discussed above.

Biochemical differences between organisms may be reflected in changes in physiology, morphology, or in both, and it therefore cannot be argued that in two related organisms which refuse to cross, physiological differences are less important taxonomically than morphological. They are, however, more difficult to work with and are thus, wherever possible, avoided by systematists.

The case described by PLOUGH shows how inability to cross may arise even within the boundaries of a single species, and it is clear that it may be induced by such changes as inversions of sections of chromosomes. Once cross-sterility or incompatibility arose there would be no need for the two races to possess the special genic combinations necessary to condition the complex sex-interest reflex. The mutants or rare allelomorphs of the genes of the sex-interest combination could then come into play for the building up, or reinforcing of other more important physiological characters of the organism. The various degrees of breakdown of the sex-interest reflex observable in the races of *Trichogramma* thus receive adequate explanation.

A further deduction may be made. If two uncrossable races, B and C are derived from a race A, and exhibit no sex-interest in each other, it is clear that in the transition to the derived races there must have been marked changes in the genetic make-up of the sex-interest reflex. As a character, the latter is vital to the existence of the species, but the genetic composition of the character may be continually changing through the superior selection pressure of other characters. Thus if a mutation from A to a, conditions a partial breakdown of some important character, a should not increase in the population,—however beneficially it contributes to a less important character, until a combination arises, (by mutation or re-shuffling) in which the damage done by mutating from A to a is compensated for. A character which is of general

importance to organisms in general should therefore be arrived at in different species, or even in different races of the same species, by widely different genic combinations. We believe that this deduction is of considerable evolutionary significance, and that the appearance of inter-sexes in the crosses of the European and Japanese Gipsy moth, reported on by GOLDSCHMIDT, can best be explained on this hypothesis.

It may be stressed that this hypothesis also serves to explain the well known phenomenon of blindness in cave-inhabiting animals. When a species is transferred to a dark environment it is no longer necessary for the intricate mechanism of the eye to be preserved inviolate. Mutants causing breakdown of the mechanism or of the organ itself would no longer be selected against, since some might be of selective value in strengthening or initiating new combinations of genes of direct physiological or morphological importance to the organism. The eyes would thus gradually become atrophied in proportion to the degree to which other characters drew upon mutant allelomorphs of the genes for the eye mechanism.

Should the existence of uncrossable biological races of *T. minutum* lead to the erection of new species? We incline to the negative view, since inability to cross, by virtue of what is known of its mode of origin, need not, though it usually does in most groups of organisms, imply profound differences in biochemical make-up. This is merely another way of stating that inability to cross may vary enormously in evolutionary significance and in taxonomic value in different groups of organisms. In *Trichogramma* it may mean little more than changes in internal chromosome organisation, and not imply very profound genic or biochemical differences. We shall for the present therefore consider *T. minutum* as a group of physiological races in the sense of the term employed by LANCEFIELD. Further work may determine whether they are entitled to specific rank.

Summary

1. Races of *Trichogramma minutum* from various West Indian islands fell into two groups in regard to colour, (a) a yellow group containing four grades and (b) a dark group.

2. Two races belonging to the yellow group were found to produce females only by parthenogenesis.

3. Constant differences in reaction to various hosts were established.
4. Crossing experiments involving a large number of combinations revealed only one fertile inter-West Indian combination, the Antigua strain proving partially fertile with the Barbados.
5. Segregation of colour was observed in subsequent generations and ultimately a new strain characterised the mass culture, possessing the colouration of the Antigua strain together with the ability of the Barbados strain to parasitise *Sitotroga cerealella*.
6. The bearing of the experiments upon evolutionary and taxonomic problems is discussed and it is concluded that in *T. minutum* it is best not to erect new species from the mutually uncrossable forms described, but to consider them as physiological races of one species, *T. minutum*, RILEY. The main reason for this is considered to lie in the fact that geographical or ecological isolation may give opportunity for chromosomal change inhibiting crossing, such change not being established as concomitant with any profound changes in genetic constitution or biochemical characteristics.

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