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THE TEMPERATURE RELATIONSHIPS OF TRICHOGRAMMA MINUTUM[1] AS A BASIS FOR RACIAL SEGREGATION

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INTRODUCTION

This study indicates only a few of the phenomena that may be investigated through the temperature responses of *Trichogramma.* Because of its numerous forms occupying a variety of habitats throughout the world, it appears to be an organism particularly suited for fundamental temperature investigations. It may also prove to be of value not only for ecological studies but for genetic or cytological investigations as well.

The morphological specific characters of *Trichogramma minutum* appear remarkably stable. In its reaction to its environment, however, the organism varies considerably. The objective of this study is to segregate such variations as are influenced by temperature.

IDENTIFICATION OF RACES

Girault,[3] in 1911, observed that in the chalcidoid genus *Trichogramma,* and *T. minutum* in particular, there existed "biological species" which he thought must be viewed as potential systematic units. From the strictly systematic standpoint, however, he did not consider them of importance. The differentiation of such races is, however, as stated by Evans,[2] of the greatest importance to the work-

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ing biologist. The importance of recognizing these "biological species," or intraspecific races, of *Trichogramma*, has in recent years greatly increased because of their possible utility in the biological control of pests.

In order to establish fully the existence of a biological race, it is not always necessary, as stated by Thorpe[9] for plant-feeding insects, to show that there is a preferred food of that particular race; that the adults have a tendency to oviposit on it; and that there is a definite tendency for members of the same race to mate together rather than with individuals of another race. Any distinctive single reaction or habit may be a basis for racial differentiation with the fundamental limitation (also pointed out by Thorpe) "That we are not really justified in considering forms . . . . as true biological races unless it has been shown that when introduced into a new environment they are relatively stable."

Recent studies by the present writer[4] on the development of *Trichogramma minutum* have shown marked differences between certain forms when reared under identical conditions in a new environment. The racial characters defined pertain to the stability of pigmentation, the length of the life cycles and the effect of changes in temperature on development. Peterson[6] in studying two color forms found, in addition to a constant difference in time required for development, an inability to inter-breed.

Under natural conditions it is probable that a correlation exists between these racial characters and certain reactions of the parasite to environmental factors. Evans[2] working with the two European forms found that one oviposited "most efficiently in the shade" while the other was "more active in a bright light." Peterson[6] noted that when the adults of the yellow form were placed on a piece of paper or upon any open surface they at once crawled toward a strong light but seldom flew or jumped any distance. Even when disturbed or touched with some object they did not fly readily. He found that the dark summer form showed in contrast a strong tendency to fly toward the light, especially when disturbed.

Evans[2] is of the opinion that "the race to be used in a certain locality should actually have been bred from a strain of the parasite found already infesting the eggs of the pest which it is proposed to control. However, since *Trichogramma minutum* is normally polyphagic, and is reared in numbers only on hosts which are rarely, if ever, attacked by it in nature, its effectiveness is not likely to be increased by obtaining the initial stock from the pest to be controlled.

It is possible that a strain from a widely different locality would work more effectively for a short season when released in numbers than the local strain which maintains itself season after season.

Peterson[6] considers that the advisability of rearing and using for liberation in the northern states a southern form or species which may not be acclimated to northern conditions is questionable. It is reasonable to suppose, however, that some northern races subjected normally to great temperature extremes would prove of wider adaptation than southern races, other ecological conditions being more or less of equal influence. The writer's observations in the laboratory of a yellow race of *Trichogramma* from Massachusetts gives some support to this theory. It should be borne in mind, however, that the effectiveness of liberations of *Trichogramma* in reducing the abundance of its hosts tends to increase directly with the decrease in seasonal temperature from that optimum for the host. Climatic conditions which result in lower host population increase the value of the per cent of parasitism.

PIGMENTATION OF ADULT AS AFFECTED BY TEMPERATURE

The various color forms of *Trichogramma* are widely distributed throughout North America. Peck,[5] in 1799, described a Massachusetts form (fig. 1) as having a pale rust color. Riley[7] reared a dark form from butterfly eggs collected in Missouri and[8] a uniform pale yellow form from the eggs of the cotton worm. Girault[3] found that those of an intensely yellow color were quite common if not usual.'' According to Peterson[6] this form appears to be typically northern. The yellow form is apparently predominant in certain localities in Massachusetts, Indiana and California. A transition form very close to the yellow form has been found only at Saticoy, California. A dark race, however, appears to the writer to be the most generally distributed from the cranberry bogs of Massachusetts, to the walnut orchards of California and the sugar cane fields of Mexico.

Since the pigmentation in the yellow race is influenced in a greater degree by developmental temperatures than that in any other race, it is here used as a basis for comparison. Within the range of its color variations are found forms indistinguishable from the color forms of the other races. The life cycle of this yellow race at a constant temperature of 77° is eight days. As this is a convenient temperature for rearing, it is used as a standard in experimental work. The 'normal limits' of its continuous development extend from 50° to 90° F. Life is at an optimum under laboratory conditions at any temperature within the 'normal limits' since, according to Chapman,[1] optimum conditions are not necessarily related to rates of processes. It is self evident that optimum conditions prevail at any period during which the environmental resistance is low enough to enable an organism to maintain its population continuously at the maximum. A high rate of development is not necessarily a factor. At a constant temperature the life cycle of the yellow race may be either as short as six days or as long as eighty.

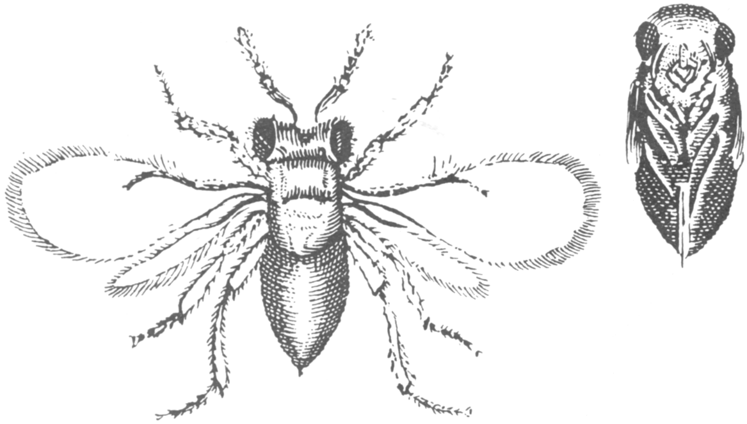

Fig. 1. Copy of woodcut of sawfly egg-parasite by Wm. D. Peck[5] published in 1799. This undoubtedly illustrates the adult and pupa of *Trichogramma minutum*. The head of the adult is tilted back showing the facial depression. Peck describes this insect as having bright red eyes, transparent wings studded and fringed with fine bristles, and antennae consisting of five articulations. In the original cut the characteristic lines of hairs on the forewings appear faintly.

The color races are separated from each other by the presence or absence of certain pigments when reared at 80° and 90° F. In every case the density of pigmentation increases with the duration of pupal development. These races in turn may be divided into life-cycle subraces, differing only by length of life cycles at identical temperatures.

All the races studied are arrhenotokous and normally winged. They were reared in the eggs of *Sitotroga cerealella* for from five to twenty successive generations. Each egg of this host usually produces one individual. The host eggs were fastened to pieces of cardboard with shellac and were exposed to the parasite for about a half hour. The complete development of *Trichogramma* took place within the eggs placed in tightly corked glass vials in an incubator in complete darkness. They were exposed to light only long enough to make the necessary observations. Only newly emerged adults were used since they do not shrink when mounted in Hoyer solution on glass slides. They were not killed before mounting. The observations on color were made against a white background.

In all the females of the races studied the eyes and ocelli were red, the club, funicle, pedicel and the distal segment of the tarsi always more or less fuscous, and the first and second tarsal segments of the middle and hind legs rarely fuscous. The general body color in all the races is yellow, but at low developmental temperatures this is apparently obscured by dark pigments. In most forms it intergrades between lemon yellow, at high temperatures, and orange yellow, at medium temperatures. The color of the males varies but little.

TABLE 1

A COMPARISON OF THE FOUR COLOR RACES GIVING THE RACIAL COLOR DIFFERENCES AT VARIOUS DEVELOPMENTAL TEMPERATURES

Temperature, degrees Fahr.	Yellow race (Massachusetts, Illinois California)	Transition race (California)	Gray race* (Georgia, Louisiana, California)	Dark race (Mexico, Massachusetts)
90	Yellow	Yellow	Abdomen fuscous. Yellow body pigment often absent in both males and females.	Abdomen fuscous. Body pigment orange yellow.
84	Yellow	Abdomen faintly fuscous	Orange yellow pigment always present	Coxae fuscous
82½	Yellow	Abdomen fuscous	Coxae fuscous	Coxae fuscous
77	Yellow	Abdomen fuscous	Coxae fuscous	Coxae fuscous
70	Abdomen fuscous	Coxae fuscous	Mesothorax fuscous	Mesothorax fuscous
64	Coxae fuscous	Mesothorax fuscous	Mesothorax fuscous	Mesothorax fuscous
59	Coxae fuscous	Mesothorax fuscous	Mesothorax fuscous	Mesothorax fuscous
50	Mesothorax fuscous	Mesothorax fuscous	Mesothorax fuscous	Mesothorax fuscous

* In the gray race from Louisiana, a small percentage of the males are wingless dwarf forms, .31 mm in length. Harold Compere, of the Experiment Station staff, examined them carefully and found the tegulae to be lacking. Occasional collections have been made in which a few specimens of the gray form have appeared at room temperature.

Table 1 presents the variation in pigmentation observed in the females of four color races at various developmental temperatures (figs. 2, 3, 4). In each case the amount of pigmentation is noted as it appears at decreasing temperatures.

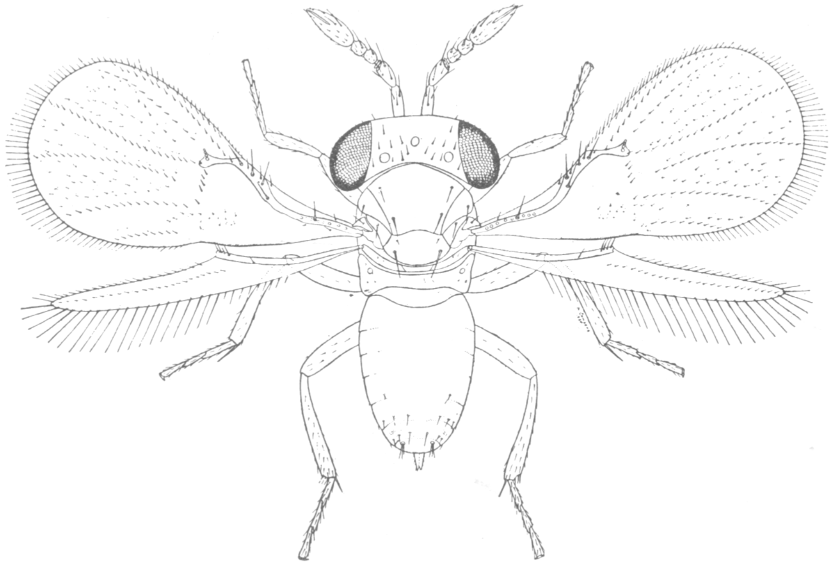

Fig. 2. *Trichogramma minutum.* Abdomen and thorax yellow without dark pigmentation. The developmental temperatures which bring about this pigmentation in the different races are as follows: 70° to 90° F for the yellow race, and 90° for the transition race.

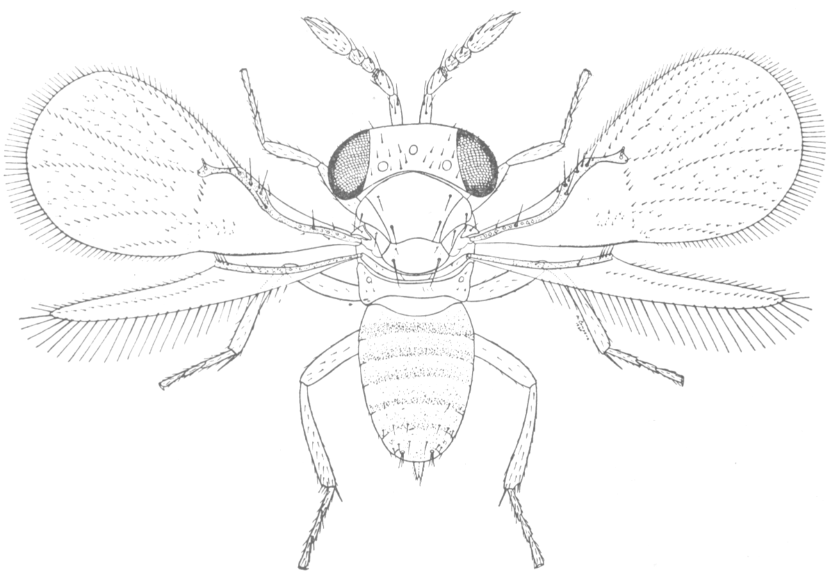

Fig. 3. *Trichogramma minutum.* Dorsum of abdomen with dark pigmentation. The developmental temperatures which bring about this pigmentation in the different races are as follows: 59° to 70° F for the yellow race; 70° to 90° for the transition race; and 77° to 90° for the dark and gray races.

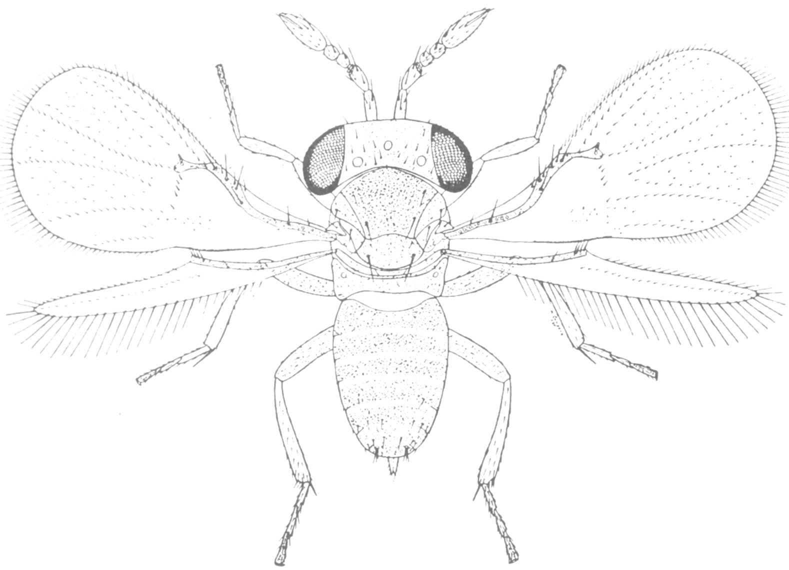

Fig. 4. *Trichogramma minutum.* Abdomen and thorax with dark pigmentation. The developmental temperatures which bring about this pigmentation in the different races are as follows: 50° F and below for the yellow race; 64° and below for the transition race; and 70° and below for the dark and gray races.

RATE OF DEVELOPMENT AS AFFECTED BY TEMPERATURE

The rate of development varies with the temperature. The increase within the normal limits which accompanies the increase in temperature probably can be approximated by Vant Hoff's law to a greater degree than in any other insect so far investigated. The rate varies, however, for each stage of development. In the normal cycle the ratio of the different stages (egg, larval, prepupal and pupal) is approximately as follows: 1: 5: 6: 12. Roughly, the rate of development for a complete life cycle is doubled by an increase of 10° F within a temperature range between 50° and 80°.

In the following experiments three color races were used; a yellow strain from Massachusetts, a gray strain from Louisiana, and a dark strain from Mexico. The purpose of these experiments was to determine the effect of sudden changes in temperature on the development of each stage and the life cycle as a whole, and also to determine the stage at which the parasite could be placed in cold storage for the longest period without injury. Only constant temperatures were used.

Experiment 1.—The parasites were reared at a temperature of 77° F except that at the end of every 24-hour period about 200 parasites of each race were placed, for a ten-day interval, at a temperature of 59° F. As a result the life cycle is prolonged seven to nine days. Those changed after the sixth day emerged while at the lower temperature. The yellow race had the shortest life cycle and the gray race the longest. The life cycles resulting from the change at each daily age of each race is as shown in table 2.

TABLE 2

A Comparison of Life Cycles of Three Color Races Reared at a Constant Temperature of 77° F, Except for a Ten-Day Interval at 59° F, at Different Ages

Age when placed at 59°F	Length of yellow race cycles	Length of dark race cycles	Length of gray race cycles
1 day (larva)	15 days 4 hours	15 days 2 hours	16 days 12 hours
2 days (larva)	15 days 4 hours	15 days 12 hours	16 days 12 hours
3 days (prepupa)	15 days 12 hours	16 days 12 hours	16 days 12 hours
4 days (prepupa)	15 days 12 hours	16 days 7 hours	16 days 12 hours
5 days (pupa)	15 days 18 hours	16 days 4 hours	16 days 12 hours
6 days (pupa)	16 days	17 days	16 days 6 hours

There was less variation in the effect on the different stages of the gray race than on either of the other two. The gray race developed slowest at 59° F, especially in the earlier stages. After the fifth day, however, it developed relatively faster.

This exposure to a lower temperature had no effect on the coloration of the yellow race until it was three days old, or in the prepupal stage. Upon emergence, the adult form of those subjected to 59° F at this age showed faint fuscous markings on the dorsum of the abdomen.

When the exposure occurred on the fourth day, however, the pigmentation was very distinct. It is evident that the length of life cycle is not necessarily correlated with the degree of pigmentation. The fuscous coloration of the adults is determined only during the pupal stage. An exposure to 59° F for about ten days is required before a change in pigmentation is very noticeable.

Experiment 2.—This experiment was conducted like experiment 1, except that the parasites were subjected to a temperature of 50° F for an interval of 24 hours. The resulting increases in length of life cycles were as shown in table 3.

The dark race at four days suffered a mortality of practically 100 per cent. The parasites died in the prepupal stage when the white urate bodies were distinctly visible.

TABLE 3

EXTENSION OF LIFE CYCLES OVER THE NORMAL CYCLE OF EACH RACE WHEN REARED AT 77° F AND SUBJECTED FOR 24 HOURS AT 50° F, AT DIFFERENT AGES

Age when placed at 50°F	Prolongation of each cycle of yellow race	Prolongation of each cycle of dark race	Prolongation of each cycle of gray race
5 hours (egg)	1 day	1 day minus 9 hours	1 day minus 4 hours
1 day (larva)	1 day	1 day minus 3 hours	1 day minus 3 hours
2 days (larva)	1 day	1 day minus 8 hours	1 day minus 2½ hours
3 days (prepupa)	1 day plus 1 hour	1 day minus 7 hours	1 day
4 days (prepupa)	1 day plus 1 hour	1 day plus 6 hours	1 day
5 days (pupa)	1 day plus 1 hour	1 day plus 6 hours	1 day plus 5 hours
6 days (pupa)	1 day plus 4 hours	1 day plus 6 hours	1 day
7 days (pupa)	1 day plus 12 hours	1 day plus 3 hours	1 day plus 5 hours
8 days (pupa)	1 day plus 1 hour	1 day	1 day

In each race the slowing up of development was greatest during the pupal period. The different stages of the yellow race varied the least in their reaction to sudden changes in temperature except on the seventh day, when in the late pupal stage a marked increase in length of cycle occurred. This may be an adaptation to a northern climate tending to prevent emergence during the fluctuating temperatures of early spring when no hosts are available. When remaining at a constant temperature of 50° F, however, this race develops slowly but continuously.

In its reaction to temperature changes, the dark race (tropical Mexican) showed the greatest variation between the early and late stages of development.

The upper limit of normal development is highest in the yellow race, as indicated in table 4.

TABLE 4

COMPARISON OF LIFE CYCLES OF TWO COLOR RACES AT HIGH TEMPERATURES

Race	90.5° F	89.6° F	83.3° F	78.8° F
Yellow race (Massachusetts)	6 days	6 days 10 hours	6 days 18 hours	8 days
Dark race (Mexico)		6 days	7 days 2 hours	8 days 7 hours

The life cycle for each race at its maximum effective temperature is six days.

Experiment 3.—In this experiment the yellow race only was used. It was conducted in the same manner as experiment 2, except that the exposure to 50° F varied for each age at which the change was made.

TABLE 5

EXPOSURE TIME AT DIFFERENT TEMPERATURES AND THE EFFECT ON THE LIFE CYCLE OF THE YELLOW RACE

Time at 77° F	Time at 50° F	Time at 77° F	Prolongation over 77° F life cycle	Additional prolongation*
5 hours	9 days	6 days 20 hours	7 days 20 hours	2 days minus 2 hours
1 day	8 days	6 days 12 hours	7 days 12 hours	2 days minus 9 hours
2 days	7 days	5 days 12 hours	6 days 12 hours	2 days minus 4 hours
3 days	6 days	4 days 12 hours	5 days 12 hours	2 days minus 12 hours
4 days	5 days	4 days	5 days	2 days
5 days	4 days	3 days	4 days	2 days
6 days	3 days	2 days 3 hours	3 days 3 hours	2 days minus 3 hours
7 days	2 days	1 day 12 hours	2 days 12 hours	2 days minus 12 hours

* Due to parasites being frozen in ice for 1½ days after removal from 50° F.

This indicates that the prepupal and late pupal periods are least affected by freezing temperatures.

Experiment 4.—In this experiment the yellow race was subjected to a temperature of 19°–20° F for an interval of six hours.

TABLE 6

EFFECT OF SIX-HOUR EXPOSURE TO 19°–20° F AT DIFFERENT AGES ON LIFE CYCLE OF PARASITES REARED AT 77° F

Age when placed at 19°–20° F	Prolongation of life cycle	Age when placed at 19°–20° F	Prolongation of life cycle
1 hour	1 hour	4 days	2 hours
7 hours	7 hours	5 days	9 hours
1 day	7 hours	6 days	3 hours
2 days	2 hours	7 days	5 hours
3 days	4 hours		

In this case the prolongation was greatest during the early larval period and early pupal period.

In order to ascertain the life cycle differences between temperature sub-races, the following test was used as a standard: At the beginning of the seventh day of a 77° F life cycle, the parasites were transferred to a temperature of 59° and allowed to emerge there. The life cycles of the different sub-races then showed the greatest amount of difference in a short length of time. The life cycles varied from 12 to 20 days.

The races subjected to this test are listed below, the shortest first and the others as their life cycles lengthen: yellow (Massachusetts), dark (Mexico),[3] gray (Louisiana), gray (California), dark (Massachusetts).

A yellow (California) and a transition (California), were not subjected to this test. Observations on their cycles, however, indicate that they are intermediate forms.

SUMMARY

There are at least four races of *Trichogramma* that can be differentiated by color when reared at identical temperatures. At certain dissimilar temperatures, however, they are indistinguishable one from the other.

A yellow race from Massachusetts is less responsive to variations in temperature, has a shorter life cycle and develops in a normal manner at a higher temperature than the dark race from tropical Mexico.

The relative differences between the life cycle sub-races is constant for certain temperatures, but the differences may vary either directly or indirectly with the temperature.

The amount of pigmentation in the adult is determined by the duration of exposure to low temperatures during the early pupal period. The slowing up of development by exposure to low temperatures is most marked in the pupal stage. The prepupal and late pupal period are least affected by freezing temperatures.

Variations in temperatures between 59° and 77° F do not produce an acceleration or lagging in the primary reaction of developmental processes to different temperatures.

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[3] When reared at 59° F, the strain from Mexico has the longest cycle.

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The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.

5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.

6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.

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