A STUDY OF THE FACTORS GOVERNING THE SEX RATIO IN *CHELONUS ANNULIPES* WESM.

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A BRACONID PARASITE OF THE EUROPEAN CORN BORER

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FOREWORD

The work of the writer was transferred for the winter of 1934-35 from the Dominion Parasite Laboratory, Belleville, Ontario, to Macdonald College, where work on this problem was commenced. This transfer seemed advisable in order that use might be made of the library facilities of the College for a search of the literature on the subject and also to make possible the investigation of certain aspects of the problem, the apparatus for which was not available at the Belleville laboratory.

In the rearing of Chelonus annulipes, as in other internal parasitic insects, one must also rear the host and since the host requires green food, it is necessary at all times, except summer, to grow the food. To have a continuous supply of parasite, host, and food on hand is a problem of some size in itself. Many of the details of the handling of the parasite and the host were developed by the writer at the Belleville laboratory but are not described herein, being only indirectly associated with the particular problem.

During the time the work was in progress at Macdonald College a difficulty of a serious nature developed. The fungus Beauvaria bassiana (Bals) Vuill., appeared in the host material, and in a short time made such inroads into it that rearing of the host had to be discontinued. This necessitated the procuring of host eggs from the Belleville laboratory and returning the parasitized material there for rearing which curtailed somewhat the amount of work it was possible to do with Chelonus at Macdonald College. In order that full advantage be taken of the facilities provided a stock of Microbracon brevicornis was secured, and some phases of the same problem with this parasite were studied. This work is also reported herein.

A study of the cytological aspects of sex determination in Chelonus was not attempted, the work being confined to experiments with the various environmental factors which might influence the sex ratio in the laboratory. Some of the cytological aspects of sex determination are discussed briefly, but only with the idea of clarifying some of the experimental data and not in the hope of making any contribution to this very difficult subject.

The writer wishes to acknowledge his indebtedness to those who made the transfer of his work possible, particularly to Dr. G. H. Barton and Dr. J. M. Swaine, of the Department of Agriculture, and to Dr. Arthur Gibson and Mr. A. B. Baird, of the Entomological Branch. He also wishes to express his thanks to Dr. W. H. Brittain, Dr. E. M. DuPorte, and Mr. J. McB. Cameron, for many courtesies while he was at Macdonald College; particularly to Mr. Cameron for assistance in the assembling and use of apparatus. Mr. Baird and other members of the laboratory staff at Belleville were most helpful at all times. Particular mention should be made of Miss L. M. Parsons for her great care in carrying out

instructions in the rearing of a very great amount of material. Without this degree of co-operation little could have been accomplished.

TABLE OF CONTENTS

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INTRODUCTION

In the introduction and attempted establishment of imported insect parasites of insect pests, it becomes necessary or desirable, at certain times and with certain species, to breed numbers of them in the laboratory. In practically all cases where breeding of hymenopterous parasites has been attempted, one of the chief difficulties has been that of securing a ratio of females to males comparable to that occurring in nature, where in this order the females are usually in excess of the males. Since, in most of the parasitic Hymenoptera so far studied, males will fertilize more than one female, the success of laboratory breeding operations is in proportion to the number of females which can be reared. The desirability of investigating the factors governing the production of females at once becomes apparent. Any contribution to the knowledge of the operation of such factors would be of considerable value to biological control work.

The parasite _Chelonus_ _annulipes_ was obtained from Italy and liberated as a parasite of the corn borer. Experimental liberations in small numbers indicated that it might be very successful under Ontario conditions. It could be obtained from Europe only in small numbers, and laboratory propagation was attempted to make larger numbers available for wide distribution. Work on this species was started in 1930 following technique suggested by the United States Corn Borer Laboratory, Arlington, Mass., but the rate of increase was unsatisfactory and a very high percentage of males was produced. Experimental work conducted by the writer (1) during the following three years resulted in a great improvement in the ratio of increase due to a marked increase in the parasitism secured. The percentage of males, however, remained abnormally high, and constituted the chief limiting factor in the propagation of the species in large numbers. Since field work in the intervening period still indicated the desirability of wider distribution of the species, it seemed desirable to investigate more thoroughly the factors influencing the sex ratio.

For the sake of clarity, it may be well at this point to indicate briefly some facts about the taxonomic position and biology of *Chelonus annulipes*. The species was first described by Nees (2) in 1813 as *Sigalphus sulcatus*. In 1818 Nees (3) removed it to the genus *Chelonus* which had been erected by Jurine in 1801 (4). In 1835 it was renamed *Chelonus annulipes* by Wesmael (5). No subsequent changes in its taxonomic position have been made. All members of this genus and of the very closely related genus *Ascogaster* are primary parasites of lepidopterous insects, and, in so far as the life histories have been worked out, are of similar habits. Although their parasitic nature was long known, the fact that they oviposited in the egg of the host was not discovered until it was noted in *Chelonus texanus* Cress. by

Pierce and Holloway (6). Pyrausta nubilalis appears to be the normal host of C. annulipes as no other hosts were found for it in Europe by Vance (7).

The females deposit their eggs in the developing embryos of the host. Only one parasite can complete its development in one host individual, but the parasites will, and frequently do, lay more than one egg in each host egg. When the host hatches, the parasite is in its body. The parasite remains in the host larva until the latter is in the fourth instar when it leaves the host and spins a silken cocoon. The average life cycle in the laboratory (80°F.) is as follows: egg stage 22 to 26 hours, larval stage 18 to 30 days, pupal stage (time in cocoon) 5 to 6 days. Mating may take place immediately on emergence and oviposition shortly afterwards. Unmated females are arrhenotokous.

Few phases of biological research have received so much attention as that of sex and few have produced such rich rewards in knowledge. But much is left to be determined, many of the "how's" have been explained but many of the "why's" remain obscure. The ultimate is probably to be found in the reason for the distinction of the sexes which may be intimately tied up with the secret of life itself. The fact that the lower fringe of the organisms which exhibit sex is being continually extended to include simpler and simpler forms impresses one with the universality of sex and its importance in the whole complex of life. To review the literature covering all this field would be well nigh impossible and is outside the scope of this paper. Comment will be limited to the mere mention of the most important landmarks on the general problem, with more particular reference to that part of the literature dealing with sex in the Insecta and more especially in the order Hymenoptera.

Where papers were in languages with which the writer is not acquainted, reliance had to be made on abstracts or translations by others, but, wherever possible, original papers were secured and read.

When man first began to speculate on what factors determined that one creature should be born male and one female is not recorded, but it probably occurred very early in the history of the race. Many theories have appeared, and some of these still persist in the lay mind. When one considers the complete absence of cytological evidence there was until very recent times, it is easy to excuse those who advanced these theories. One early theory was that one ovary produced males and one females. This was disproved by the fact that animals could still produce males and females after the removal of one ovary. Another theory was that the resultant sex depended on the time during the period of oestrus when copulation took place. This, for most cases, has been disproved, but in some cases there is evidence that time of fertilization has an effect on the sex ratio of the resultant offspring. Late fertilization in echinoderms affects sex ratio and Barnes (8) states that in Dasyneura arabis the further developed the eggs are at fertilization, the more males result.

Really scientific observation on the matter commenced with Mendel in the sixties of the nineteenth century. He suggested the possibility that sex determination might prove to be a phenomenon of heredity and segregation. Subsequent work by Castle (9, 10) seemed to prove this. He summarizes in part as follows, "We may, it seems to me, summarize our present knowledge (1909) on sex inheritance under one consistent scheme as follows: 1. Sex is not directly controlled by the environment but is determined by internal (gametic) factors. 2. The determination of sex depends upon the presence in the zygote of a factor or factors which are inherited in accordance with Mendel's law". At present, however, this cannot be accepted without reservations. If sex were a strictly Mendelian character, the zygotes of the two sexes would be produced in equal numbers and, barring pre-natal mortality, would appear in equal numbers at birth. In the majority of forms the preponderance of males is normal and constant at birth (Pelseneer) (1926) (11). Differential mortality pre-natally could account for it in certain cases, but certainly not in the majority. Pearl (1917) (12) indicates that in poultry there are consistently more females than males at birth. He investigated the pre-natal mortality from the tenth day of incubation forward and found that it was not differential as regards sex. He admits that theoretically one should be able to sex every zygote, but that practically it is not to be seriously considered, and concludes by stating that there is justification for believing that the observed sex ratio at birth is substantially the same as the initial zygotic sex ratio. There are, in addition, cases in insects where all the eggs of fertilized females have been reared, and where the sex ratio has shown inequality after adding to the less numerous sex all individuals which died.

The first notice of what was undoubtedly a sex-chromosome was made by Henking (13) in 1891. He noted that in *Pyrrhocoris apterus* half the spermatids contained a nucleolus-like body missing in the other half. In 1898 Paulmier (14) recorded a similar condition in *Anasa tristis*. In 1901 deSinety (15) described the behaviour of an unpaired chromosome in the male of a *Phasmid*, and, in the following year, McClung (16) suggested that the two kinds of chromosomes might be connected with the production of the two sexes. In 1906 Doncaster (17) traced the inheritance of sex by means of a sex-linked character in crosses of the lepidopterous insects *Abraxas grossulariata* and *A. laticolor*, and proved that, in this order, the female has two types of gametes, and is, therefore, heterogametic. Punnett (18) has demonstrated the same for fowl using a sex-linked plumage character. Here, as in the Lepidoptera, the females possess the factor in the single state, and are, therefore, heterogametic, while the males possess it in the duplex state and are homogametic. Later work has demonstrated that, in practically all forms of life, the male is the heterogametic sex and the female the homogametic, the two groups mentioned above, the Lepidoptera and the birds being the exceptions. Some seventy characters in _Drosophila melonogaster_ have been shown to be inherited in a typical sex-linked manner. In 1907 Correns (19) determined that the same held for plants, and that the pollen grains are of two kinds, while the ovules are all alike.

Crew (1923) (20) sums up the matter as follows: "The evidence which is now available proves beyond all doubt that there exists a definite relation between the gametes and the sex of the individual resulting from their union and that sex is actually (though not necessarily finally and irrevocably) determined at the time of fertilization by the sex chromosome, sex-distributing mechanism. It has been demonstrated that the pre-destination of sexual genetic constitution in the gametes is established during spore formation in plants and during gamete formation in animals".

Primarily the determination of the proportions of the sexes is made at the time of fertilization. Normally in animals in the heterogametic sex the two types of gametes are formed in equal numbers. This being true, if no other factors intervene which give an advantage to one type over the other, the primary sex ratio will be 50:50. It has been shown, however, that sexual states may be altered or reversed by appropriate changes in external factors acting through the metabolic process. This has given support to the view that this difference between the sexes is one of the rate or level of metabolism. For example, Riddle (1917) (21) has shown that the first egg of a pigeon, which it has long been known develops into a male, differs from the second, which develops into a female, in both size and water content. As the season goes on, the eggs become more and more female in type, and the females themselves, which earlier in the season were slightly male in their secondary sexual characteristics, become increasingly feminine, and the percentage of females produced increases. He was able, by changing the water content, to induce experimentally an actual reversal of the sex tendency. He concluded that sex is quantitative, and of a fluid character not representing two qualitatively distinct, mutually exclusive properties, but rather two levels in a continuous series of intergrading states. Baltzer (1914) (22) found that in *Bonellia*, the males of which are parasitic upon the proboscis of the females, those not becoming attached to the proboscis of a mature female develop into females, while those which succeed in becoming attached for a sufficient time develop into males. All grades of intersexes were produced by limiting the time spent on the proboscis. Holdaway (1930) (23) demonstrated that the sex of *Tribolium confusum* could be changed by starving the larvae for certain periods. He states, "The oscillating nature of the change in sex ratio indicates that either some forms change from one sex and back again, or that certain forms of both sexes change to the other after different periods of starvation". He concludes, "That either (1) a proportion of the population is stable, as regards its expression of sexuality, while a proportion is more easily converted to the opposite sex one way or the other, or (2) that forms of either sex may be changed to the opposite sex, but that there are gradations of sexual stability only upset after varying periods of time. The effect of nutrition on sex determination undoubtedly takes place per medium of its effect on the biochemical and biophysical state of the body fluids and through them also on the germ cells. In insects one does not consider that more than two sexes occur, but the results briefly quoted indicate that there are at least gradations in constitution which show a relation between nutritional status dependent on time of starvation and the ultimate sex expression".

Christie (1929) (24) observed that in *Mermis subnigrescens*, where the population in a single host was high, the parasites were nearly all males, whereas where the population was low or the parasites were single, they were nearly all females. It would seem that, in this species, any theory which holds that the sex is irrevocably established at the time the parasites enter the host must be abandoned, and the conclusion must be that in this case environment must have a sex determining role. The few examples cited, while not directly connected with the problem in hand, serve to indicate that sex is not irrevocably settled at the time of fertilization, and that any investigation on the numerical ratios of the sexes must take cognizance of the effects of nutritional influences on the embryonic and immature stages of the organism under examination.

Goldschmidt (1923) (25), in discussing alterations in the numerical ratios of the sexes, warns against confusing changes in the sex ratio with an actual effect on the sex-determining mechanism. Variations, arising out of the mechanism of sex distribution, may be produced experimentally in the laboratory, but, in his opinion, in the rarest cases is there a real sex determination, that is, either a directional influence on the distribution mechanism or an influence on the physiology of sex differentiation. The following are listed as such:

1. In experiments with zygotic intersexuality
2. Bonellia type
3. In parthenogenetic forms
4. Overripe eggs as in frogs
5. Hereditable mutation

Goldschmidt also warns against errors in statistics, stating that no valid conclusions can be drawn unless the fate of all the fertilized ova is known. Variations from the normal may not be due to sex-determining factors, but to selective elimination of one sex at some stage previous to that in which observations are being made. In the majority of forms, it may be taken for granted that fertilized ova will be potentially male-producing and potentially female-producing in almost equal numbers. But, in the Hymenoptera, in which we are particularly interested, the matter is complicated by parthenogenesis, by which unmated females will produce one sex only, and mated females may produce varying numbers of both sexes dependent on whether or not the eggs are fertilized. The complication arises from a lack of the information which Goldschmidt (25) says is necessary, namely, the ratios of fertilized and unfertilized eggs.

As early as the time of Aristotle, the phenomenon of parthenogenesis was suspected in the honey bee, but he had difficulty in explaining the three kinds of bees in the hive. He concluded that the queens produced queens and workers without mating, and that the workers produced the drones which, in turn, produced nothing. It was not until two thousand years later that a tenable theory to explain this phenomenon was stated by Dzierzon. Dzierzon was a curé of Carlsmarkt who first published his ideas in an obscure apicultural journal in 1845, and later (1848) (26) in an enlarged and more scientific form. His theory fits in with the facts as they are known today; that unmated females whether queens or workers produce only males, but that mated queens produce males and females, the latter being derived from fertilized eggs and the males from unfertilized eggs.

Von Siebold (1856) (27) introduced the subject to zoologists and somewhat clarified Dzierzon's statement. Parthenogenesis in other forms was known at an earlier date by Goedart (1667), Saint Blanchard (1896), and Albrecht (1706), but their observations were not made under strict technical conditions. They referred to the phenomenon as "Lucina sine concubitu". Reaumur (1742) (28), suspecting that aphids reproduced without mating, conducted experiments to prove it, but these were unsuccessful. Charles Bonnett (1745) (29), inspired by Reaumur, in careful experiments, demonstrated the fact of parthenogenesis in aphids. Before long, further demonstrations were brought forward by other workers. It was natural that attempts should be made to explain these occurrences. Trembley, cited by Bonnett (29), thought that one fertilization might last through several generations, but von Siebold demonstrated that these forms had no receptacle seminalis while that those which laid eggs had. Some naturalists of this period thought that forms which reproduced without mating were hermaphroditic, while others thought that reproduction was by budding. Huxley (1858) called the parthenogenetic ova "pseudova" and the organ forming them "pseudovaire". Claus (1864) (30) established definitely the identity of the parthenogenetic egg. Biological studies like the above established the fact of parthenogenesis, but it remained for cytology to bring forth explanatory data.

Weismann (1886) (31) observed that parthenogenetic eggs gave off a single polar body, while fertilized eggs gave off two. The same was demonstrated for aphids by Blochmann (1887) (32), and holds for many forms, but is not a universal rule.

The arrhenotokous type of parthenogenesis, that in which only males are produced from unfertilized eggs, is most common in the Hymenoptera. Herting (1912) (33) calls it parthenogenesis of the hymenopterous type. Thelyotokous parthenogensous, wherein only females are produced from unfertilized eggs, occurs in the Hymenoptera but is not so common as the former type. In the Cynipidae, cyclical parthenogenesis alternates with sexual reproduction. Doncaster (1910, 1911, 1916) (34).

Few subjects in biology have more extensive bibliographies than that dealing with the hive bee, and particularly that dealing with reproduction. This literature is important in the present case, in that reproduction in the bee is similar to that of the parasitic Hymenoptera. As mentioned earlier the first important landmark was Dzierzon's enunciation of his theory. He believed that in the beginning all eggs were alike, but at the moment of passing the seminal receptacle they might or might not be fertilized; those fertilized produced females and those unfertilized produced drones. The correctness of this theory has also been proven for many other Hymenoptera. Paulcke (1899) (35) examined large numbers of the eggs of honey bees, and in eight out of twelve eggs laid in worker cells found spermatazoa, and none in eight hundred eggs destined to become drones.

Several workers have secured results which would seem to upset Dzierzon's theory. Male offspring, having only one parent, should have only maternal characters. Lowe (1867) (36) and Perez (1878) (37) secured males which had paternal characters. Difficulty of controlling the mating of bees made these earlier attempts at proof difficult, and it was not until Newell (1914) (38) carried on experiments with pure lines of Carnolian and Italian bees in a desert region, where chance matings would be practically eliminated, that the theory was completely vindicated. In his first cross, all males bore only maternal characters, while females were bi-parental in character. Whiting (1928) (39), working with Habrobracon, shed light on the reason for some of the confusing results in earlier work. He found that, when widely separated races were crossed, diploid males were produced, but that these were usually sterile. This may have been the case with some of the earlier experiments with bees.

If fertilized eggs produce females and unfertilized eggs produce males, and the chances of survival are similar for both sexes, the sex of the offspring will be in the proportions in which fertilized and unfertilized eggs are laid. The question is what influences the fertilization or non-fertilization of the eggs. Here again we refer to the very extensive work on the honey bee. Is the fertilization controlled by the "will" of the female or is it the result of environmental conditions? To assume that the female "wills" to fertilize eggs also assumes that she has the necessary apparatus to give effect to her will.

Chesire (1886) (40), Breslau (1905) (41), and Adam (1912) (42) have demonstrated that the female bee has organs which might perform this function. A spermatheca receives enough seminal fluid to last the lifetime of the queen. The emission of this through the spermathecal duct is controlled by special muscles of the latter structure called a sperm pump. Adam (1912) (42) believed that about ten spermatazoa were passed out at each time, and that the micropyle of the egg is placed in contact with the opening of the canal. Breslau (1905) (41) estimated that one hundred spermatazoa were ejected for each egg. One cannot but wonder what becomes of the spermatazoa not used at each fertilization, whether they are lost or are pumped back into the spermatheca by a reversa of the motion which brought them out. Unless some disposal were made, the vagina under such conditions would soon swarm with spermatazoa, and any egg which passed through it could not help but be fertilized, regardless of the will of the female. Von Siebold (1856) (27) and Leuckart (1858) (43) believed that the size of the cell, on which the queen finds herself, determined by reflex action whether or not seminal fluid is passed. Perez (1878) (37) cites an experiment by the bee keeper Drony, in which only worker cells were presented at the season when drones are produced in quantity, and only drone cells at the season when workers only are produced The queens laid drone eggs in worker cells and vice versa in spite of their size. Old bee keepers attributed occurrences of this sort to "errors of instinct", but they seem to indicate rather definitely that size of cell has little to do with the fertilization or non-fertilization of the eggs.

In the Hymenoptera, a wide variation in the organs of reproduction is present. In the Tenthredinidae, there is no distinct spermatheca but simply an evagination of the vagina. It is inconceivable that, in such cases, either the will of the female or the environment could have much influence on the fertilization of the eggs of a mated female. Some workers assume that in these lower forms practically all the progeny of mated females are female. Genieys (1925) (44) studied in some detail Habrobracon brevicornis, and found that, in a mated female, the first few eggs gave mostly males, while later eggs gave mostly females, except that at the end of the oviposition period males again predominated. Vandel (1930) (45) comments on the findings of Genieys as follows: "There exists, therefore, it seems, a mechanism capable of assuring the insemination of the eggs or on the contrary of hindering it". This seems a rather dangerous generalization, for the lack of females in the late offspring may be due, as in the case of the bee, to the depletion of the seminal fluid after a long period of egg-laying. Descy (1924) (46) studying the mason bees found that all the eggs destined to be males were laid in small cells, and all destined to be females were laid in large cells. He believed that, when a female has not been inseminated, she builds small cells and lays unfertilized eggs in them, whereas, when the spermatheca is full, the female constructs large cells and lays in them fertilized eggs. A similar condition occurs in some of the parasitic Hymenoptera. Chewyreuv (1913) (47), working with *Exenterus* sp. and *Campoplex* sp., exposed large and small larvae for parasitism. He found that, with large host larvae, the progeny of the parasites were predominantly female, while, with small host larvae, they were mostly male. Chewyreuv concludes that this matter of females coming from larger hosts is similar to that of the queen bee laying unfertilized eggs in large cells, or, in other words, that the females are guided by the size of host or cell as to whether they do or do not fertilize the eggs they are laying. Holdaway, and Smith (1932) (48), working with *Alysia manducator*, found that males usually emerged from small host puparia and females from large puparia. In their discussion of their observations, they state "If they (males) were from haploid eggs derived from unfertilized females, one would have to conclude that unfertilized females restricted their attention to the smaller hosts. If they were from haploid eggs from fertilized females, one would have to conclude that there was a mechanism for controlling the fertilization of the egg to the size of the host". They suggest the possibility that some of the males may be diploid, indicating that, in their opinion, quantity of food as regulated by size of host played a part in the determination of sex. Seyrig (1935) (49), working in Madagascar with *Pimpla maculisaposa* and *Echthromorpha hyalina*, found that females emerged from large hosts and males from small hosts, a variety of host species being involved. He believes that since males are the more numerous, most of the eggs laid are unfertilized, that large females have a greater chance of being fertilized, because they secrete a greater amount of the odiferous substances which attract the males, and that these large females, capable of producing more females, follow the general rule and return for oviposition to the same species from which they emerged, the unmated smaller females returning to the smaller species from which they emerged. Vandel (1935) (50) points out that this theory is scarcely tenable since the same occurs where only one species of host is involved, mostly males emerging from small hosts and females from large individuals.

Brunson (1937) (51), working with Tiphia popilliavora Roh. on Popillia japonica Newm., seems to have covered some points neglected by others in experimenting in this matter of size of host and sex of parasite. He found, as others did, that large or third instar hosts usually gave female parasites, while second instar hosts gave males. He found this to hold whether both sizes were present or not, but that where both were exposed the parasites preferred the larger hosts. He states moreover that "Definite proof, that the female parasite has the ability to vary the sex of her progeny at the time of parasitization of the host larvae of different instars, was obtained, when parasite eggs placed by fertile females on second instar host larvae were transferred to third instar host larvae, and eggs placed on third instar larvae were transferred to second instar host larvae". The resulting parasites were of the same sex, as if they had remained on the larvae on which they were laid.

Vandel (1932) (52) observed that, in some parasites, where the number emerging from one host varies from single individuals to many, when the parasite is solitary it may be a male or female, but, when several parasites co-exist in the same host, the male sex usually predominates. From this, he concludes that the number of parasites contained in the same host does not influence directly the mechanism of sex determination, but the numerical ratio is influenced indirectly by selective elimination of the female sex. This elimination is due, in his opinion, to the greater demands of the females in regard to food, and that they, being more sensible to lack of proper nourishment, die first.

STATEMENT OF SPECIFIC PROBLEM

As stated earlier, with Chelonus annulipes and other hymenopterous parasites, the problem is to secure, in their laboratory propagation, females in percentages comparable to those obtaining in nature. To form a basis for judgment of the matter in Chelonus, it is necessary to examine the available data on the sex ratio of the parasite in its native home and also in the recoveries in areas where it has been introduced. Vance (1932) (7) states that, under normal field conditions in Europe, in both first and second generations the sexes are approximately equal. The earliest liberations of this species in Canada were made from parasites imported in the immature stages, reared out, and liberated as adults. Later liberations have been largely from laboratory reared material, although there has been some mixing of imported and laboratory material. The information available in regard to the liberations and their progeny of the first generation as represented in recoveries is stated in Table I.

Table I

Year	Location	Source of Liberated Material	Recoveries		
			♂	♀	% ♀
1931	Chatham	Imported	71	85	54.4
1933	Belleville	Laboratory	42	20	32.2
1934	Prince Edward	bred	14	3	17.6
1935	" "	" "	196	80	28.9
1936	" "	" "	3	2	40

It will be noted that the only time when the sex ratio of the recovered parasites was comparable to that of the parasite in its native home was when the adults liberated were from direct importation. Recoveries from laboratory-bred material were consistently lower in females.

When laboratory breeding work was first initiated in 1930, mated females from imported stock were used for the first generation; subsequently spring breeding stock was secured from various sources. Table II indicates the sources of breeding material and the numerical ratios of the sexes secured in the progeny.

Table II

Year	Source of Breeding Stock	♂	♀	% ♀
1930	First Generation, Imported	143	152	51.5
	Second Generation, Laboratory bred	237	151	38.9
1931	Mixed - Recovered and Imported	496	251	33.6
1932	" " " "	1402	1131	44.6
1933	Recovered (Laboratory Garden)	3030	1491	32.2
1934	Laboratory Bred Stock	8734	3198	26.7
1935	" " "	7139	2746	27.7
1936	" " "	3881	1982	33.8
1937	" " "	9346	5222	35.8

It will be noted that the results in the laboratory rearing are consistent with those for liberated material. Stock only one generation removed from imported material shows a sex ratio comparable to that which obtains in the native home of the parasite, but, where laboratory-bred material has been used as the basis of stock or for liberations, the resulting progeny have shown a low percentage of females.

There appears a slight rise in ratio of females in 1936 and 1937. For breeding material during these two years an effort was made to breed from females which produced large numbers of females in their offspring. Also in 1937 a different host insect was used, Ephestia kuehniella. Reference will be made to these aspects of the problem later.

If the species required fertilization before development of the eggs could take place, the search for factors altering the sex ratio could be confined to those influences which might eliminate one sex and allow the other to develop. But the fact that the species is parthenogenetic makes it necessary to also investigate the influences which determine whether or not the eggs of mated females are fertilized. This makes the problem fall naturally into two parts. The first part concerns all those influences which might have an effect on the numbers of fertilized and unfertilized eggs which are laid. The second part of the problem concerns any factors which might act on the parasitized host larvae or the contained parasite larvae in such a way as to produce conditions more unfavourable to one sex of the parasites than to the other. These factors are summarized briefly below:

Factors acting on adults from time of emergence to completion of oviposition.

1. Light (quantity and quality)
    - (a) At time of mating
    - (b) At time of oviposition
    - (c) During storage and feeding
2. Temperature and humidity
    - (a) At which males are held prior to mating
    - (b) At which females are held prior to mating
    - (c) At time of mating
    - (d) At time of oviposition
    - (e) Between oviposition periods
3. Food of adults
    - (a) Kind
    - (b) Presence or absence
4. Crowding
    - (a) During storage
    - (b) At time of mating
    - (c) At time of oviposition
5. Age of females
    - (a) At time of mating
    - (b) At time of oviposition
    - (c) Duration of periods between oviposition periods
6. Age of males at time of mating
7. Age of host
8. Size and condition of host
9. Number of eggs laid each day

Factors acting after oviposition.

1. Selective elimination of one sex
    - (a) By scarcity of food where parasites are gregarious (*Microbracon brevicornis*).

(b) By one sex requiring more food than another for completion of its development, thus killing more hosts before it matures and causing its own death.
(c) By a more rapid rate of development, either embryonic or post-embryonic, of one sex which gives it an advantage over the other sex in a competitive struggle for food or space.
(d) Early host mortality, whereby the faster developing sex may survive, the slower may be cut off by death of the host before its development is completed.
(e) Temperature at which host larvae are reared.

2. Change of sex.

(a) By nature of the food
(b) By quantity of food
(c) By temperature and humidity

3. Heredity.

It has not been found possible to investigate all the above factors, but what have been considered the more important ones have been studied. Data gathered during the rearing of large numbers for liberation shed additional light on some aspects of the problem and where this occurs these data have been used.

PROPORTION OF FERTILIZED EGGS LAID

The desirability of knowing in what proportions fertilized and unfertilized eggs are being laid is important since definite knowledge on this point would limit the work to be covered to one of the two main divisions as indicated earlier. To be of use, however, this information would have to be accurate. The problem consists in finding why there are approximately 30 to 35 per cent females in the laboratory as against 50 per cent in nature. Therefore, errors of from 5 to 10 per cent would make information gained on this point of little value. Pearl (12), working with as large an organism as the domestic fowl, states in this connection, "the determination of the sex potentialities of the egg at the time of fertilization is scarcely to be seriously considered". In the hive bee, in eggs known to be destined to produce females, Paulcke (35) found spermatazoa in eight out of twelve eggs. Gatenby (1917) (53) in doing the embryology of *Trichogramma evanescens* only discovered evidences of fertilization in a few cases. It became apparent that cytological methods would not yield results of sufficient accuracy to be of use in this case. However, an attempt was made along other lines to shed some light on this phase of the problem.

As in other parthenogenetic forms, fertilization is not necessary to initiate development of the egg. It is known also that, in most parasitic Hymenoptera, males emerge before females. This is also true for Chelonus. The question arises as to whether or not this more rapid rate of development extends back as far as the egg, that is, do eggs destined to produce males hatch in a shorter period than those destined to produce females, and if so, could this differential rate of hatching be used to separate fertilized from unfertilized eggs?

A search of the literature failed to reveal any case in which the embryology of both the male and female of any Hymenopteron had been covered in its entirety. Nelson (1915) (54) quotes Petrunkewitsch (1902) (55) as indicating that with the honey bee gastrulation takes place in the drone five hours before it does in the worker egg. The time for total development is unknown. However, this seemed enough evidence of more rapid embryological development of the male to warrant some efforts to separate fertilized from unfertilized eggs by difference in hatching rate.

In some earlier histological work on the species (1934) (1), it was found that eclosion took place approximately twenty-four hours after the eggs were laid when the host eggs were kept at 80° F. This was used as a basis for further study. The method used was as follows: Host eggs were exposed for parasitism to virgin females and to mated females, and, after periods varying from 22 to 25 hours incubation at 84° F., were fixed and prepared for examination. The following procedure was used for the preparation of these eggs (van Steenburgh) (56).

Eggs killed and fixed in Bouin's fluid at 60° C. 24 hours or more.
Alcohol 50 per cent 24 hours.
Alcohol 70 per cent 24 hours.
Eggs pricked with sharp instrument to permit staining.
1/4 Methylene Blue, 50 per cent alcohol 24 hours.
Alcohol 70 per cent 24 hours.
Alcohol 95 per cent 4 hours.
Alcohol 100 per cent 3/4 hour.
To Xylol.
Mounted in Canada balsam.

Eggs treated in the above manner showed very clearly which parasite eggs were hatched and which unhatched. Figures 1. and 2. show typical samples of these eggs. An analysis of the results obtained is given in Table III. It is evident that the variation in the hatching time of the eggs laid by virgin females is such as to preclude any possibility of separating fertilized from unfertilized eggs on the basis of the time required for completion of embryological development.

Table III
Rate of Hatching

Eggs Laid by	Time Incubated	Eggs Unhatched	Eggs Hatched
Virgin Females	21.40 -- 22.00	0	3
	21.55 -- 22.10	5	5
	22.40 -- 23.20	18	8
	22.45 -- 24.00	0	2
	23.15 -- 23.35	10	0
	23.35 -- 24.15	15	24
	24.15 -- 25.30	3	6
	24.30 -- 25.30	20	43
Mated Females	21.20 -- 21.50	6	3
	21.25 -- 21.40	0	11
	21.50 -- 22.05	7	4
	22.40 -- 23.20	10	32
	23.15 -- 23.35	5	10
	23.30 -- 24.30	2	2
	23.35 -- 24.15	13	21
	24.15 -- 25.00	11	7
	24.15 -- 25.45	5	0

Figure I. Parasite egg in host egg.

Figure 2. Parasite egg and parasite in one host egg.

EFFECT OF LIGHT AT TIME OF MATING

For this experiment Microbracon brevicornis was used, lack of material preventing the use of Chelonus annulipes. The females used in the experiment and those used in the checks were all progeny of one female. The checks were mated under ordinary incandescent light, and the others were mated under a mercury arc in quartz which, as indicated elsewhere, gave practically all the effective rays of natural sunlight and was rated by the makers to give a line spectrum of 2,500 to 7,000 Angstrom units. In all other respects all females were treated alike.

No notable stimulation was evident in the mating under the mercury arc as compared with what occurred under ordinary incandescent light. Tables IV and V show the results obtained in the material mated under the mercury arc and the check respectively.

Table IV
Mated in Ultra -- Violet Light (Mercury Arc)

Female No.	No. Days	Total Eggs	♂	♀	% ♀	Female out of Female
35	8	145	36	37	50.68	5
36	10	181	27	47	63.51	5
38	9	129	26	30	53.57	5
39	8	154	82	21	10.68	5
40	3	22	5	5	50.00	5
41	9	156	21	71	77.17	5
42	7	145	81	44	35.20	5
44	7	37	11	18	62.06	5
		969	289	273	48.57	

Table V
Check - Mated in Ordinary Incandescent Light

Female No.	No. Days	Total Eggs	♂	♀	% ♀	Female out of Female
21	10	222	63	112	64.00	5
22	9	180	39	105	72.94	5
23	9	199	91	63	40.99	5
24	3	88	43	10	18.81	5
28	6	124	33	15	31.25	5
29	5	95	18	64	78.05	5
30	11	243	46	136	74.72	5
31	9	236	54	96	64.00	5
32	8	168	69	51	42.50	5
33	8	160	31	54	62.22	5
34	6	116	38	23	37.70	5
		1831	525	729	58.1	

Examination of the data reveal that any advantage in the matter of the production of females is in favour of the check in which ordinary incandescent light was used. High and low female production is evident in both groups. If any conclusion can be drawn, it would be that kind of light at time of mating was not a significant factor in determining the sex ratio.

EFFECT OF LIGHT AT TIME OF OVIPOSITION

In the laboratory all breeding of parasites is carried on under what may be considered highly artificial conditions. This is particularly so in so far as light is concerned. In winter artificial light from incandescent electric lamps is used extensively, and even in summer what daylight reaches the mating or ovipositing insects usually does so through one or more layers of ordinary glass, either in the windows or in the cages where the insects are confined. The experiments herein described were planned to determine the effect on the sex ratio of light of different frequencies at the time of oviposition.

The female parasites (Chelonus annulipes) used for this experiment were drawn from the ordinary laboratory stock. They were mated as soon as possible after emergence, which is the usual practice, and shipped by mail from Belleville to Macdonald College. Host eggs also were shipped from the laboratory to Macdonald College daily. These were parasitized under the variety of light conditions which will be described later, and returned for rearing to Belleville the same day as they were parasitized. The arrangement worked very well and no undue mortality occurred due to shipping.

The arrangement of the apparatus used in the experiment is shown in the accompanying drawing (Figure 3.).

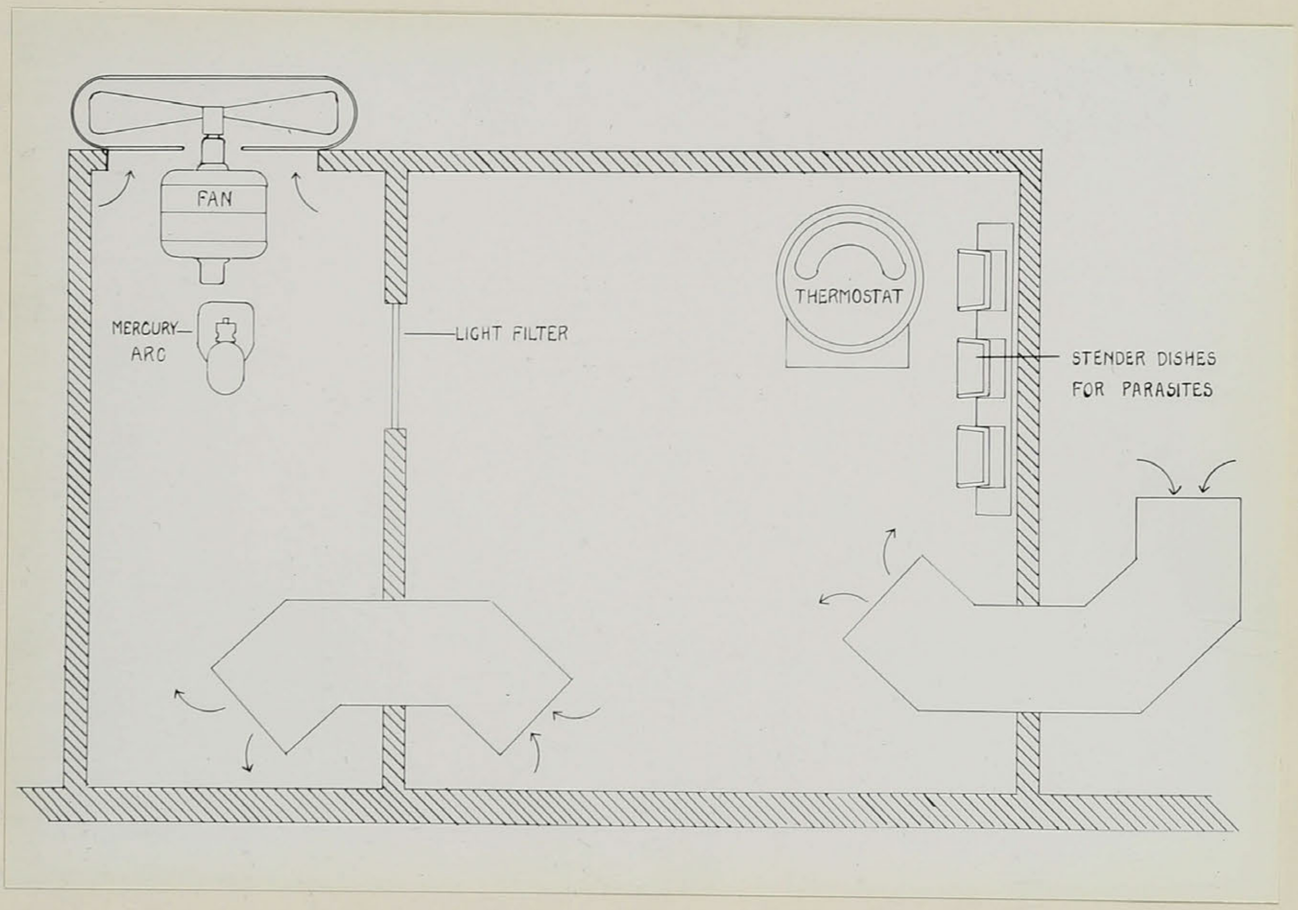

Figure 3. Arrangement of apparatus for light experiment.

The apparatus was housed in a box made of Celotex, approximately 23 inches long, 15 inches high, and 15 inches deep. Acess was secured through a door on the front. The distance from the source of light to the filter was 4 inches and from the filter to the jars, containing the experimental material, 13 inches. Considerable heat was generated by the mercury arc which, if left confined, would raise the temperature to a point where it would be dangerous to the parasites and the host eggs. To prevent this, forced ventilation was installed. Bent paper tubes with blackened interiors were placed in the end on which the material was exposed and in the partition between the two sections. A fan, placed in an opening just above the mercury arc, drew the air from the outside through the two chambers, thus keeping both from becoming unduly warm. The room in which the experiment was carried on had an average temperature of about 62° F. As it was not desirable to cool the chamber down below 80° F., a thermostat was installed near the rack which held the stender jars containing the parasite material. This thermostat, by turning on and off the fan as the temperature rose and fell in the chamber, maintained the temperature in the neighbourhood of 80° F.

Source of Light.

The source of light was a mercury arc in quartz glass. This lamp was rated to give a line spectrum of 2,500 to 7,000 Angstrom units.

Filters used.

The filters used were chosen from a set of thirteen put out by the Corning Glass Company. Since it was thought inadvisable to test all the different lights made available by these filters, those representing the four ranges from which effects on the parasites might be expected where chosen. The following combinations were used:

Naked Mercury Arc - Line spectrum 2,500 - 7,000 Angstrom Units.
Mercury Arc - No. 1 - Transmits Infra Red only - 7,000 - Angstrom Units.
Mercury Arc - No. 6 - Cuts out all Ultra Violet and transmits 75 - 80 per cent of white light falling on it - 4,000 - 7,000 Angstrom Units.
Mercury Arc - No. 9 - Transmits approximate Primary Blue 4,100 - 4,700 Angstrum Units.
Mercury Arc - No. 13 - Passes only Ultra Violet 3,656 Angstrom Units.

In addition to the above, several lots were run with no light whatever during the time the female parasites were with the eggs, but in the same environment in so far as other factors were concerned.

In order to prevent the escape of the parasites from the stender jars, it was necessary to cover them. For this purpose cellophane was chosen beacuse, of all the available materials, it passed the greatest number of rays. It also reduced the intensity only a very small amount. The following are the readings secured by the use of the photronic cell with and without cellophane:

Source	Distance	Filter	Covered With	Units Passed
Mercury Arc	18"	--	--	502
" "	18	--	cellophane	435.5
" "	18	No. 6	--	261.5
" "	18	No. 6	cellophane	242
100 watt Frosted Lamp	18	--	--	161.5
" " " "	18	--	cellophane	148.5
" " " "	10 5/8	--	"	435.5
" " " "	7 5/8	--	cotton	502

The photronic cell available was sensitive to white light only; it was, therefore, impossible to measure the intensity of the light passing through filters No. 1, 9, and 13. In the laboratory in dull weather, artificial light is used when the parasites are ovipositing. Frosted incandescent lamps are used for this purpose, and are placed at such distances from the insects as would be comparable to those indicated for the last two measurements above.

As indicated previously, the parasites used in this experiment were from the regular breeding stock. As this species is arrhenotckous, it is absolutely necessary that the females used be mated if any significant data on sex ratio are to be secured. All females used were observed to copulate, but, in order to positively eliminate all unmated females, each female was numbered, and she and her progeny of each days oviposition handled separately throughout the experiment.

The procedure for each day was as follows: Eggs were received from Belleville, and, when approximately sixty hours old, the proper time for oviposition, (Wishart and van Steenburgh) (1934) (1), were placed in the stender jars with the parasites. Two average sized masses or three smaller ones were placed in each jar with one female. The eggs were held in place by a small pin stuck into a piece of thin cork wedged into the bottom of the dish. The top of the dish was then covered with a piece of cellophane held in place by a small elastic band. Each dish was placed in a hole in the rack in the chamber and left for approximately two hours. The eggs were then removed and mailed to Belleville where each lot was reared out individually. As is the practice in the laboratory, the females were kept between oviposition periods in a cool place.

The data for the various conditions of light are summarized in Tables VI, VII, VIII, IX, X, and XI.

Table VI

Ovipositions under Mercury Arc

No.	Date	♀ No.	♂	♀
101	March 20	1	1	0
117	" 30	2	11	5
127	April 4	1	1	0
128	" 4	7	1	1
129	" 4	8	0	4
131	" 4	10	2	1
148	" 9	2	1	0
151	" 9	9	4	0
175	" 15	7	1	0
176	" 15	8	1	0
177	" 15	9	2	0
178	" 15	11	1	1
180	" 15	13	2	0
199	" 21	7	1	0
201	" 21	11	1	0
			30 71.5 %	12 28.5 %

Table VII

Ovipositions under Mercury Arc - Filter No. 13

No.	Date	♀ No.	♂	♀
109	March 27	2	5	10
121	April 1	2	1	0
137	" 6	8	1	2
138	" 6	7	14	5
156	" 11	7	7	5
157	" 11	8	5	9
158	" 11	9	9	5
187	" 17	7	7	0
188	" 17	8	2	2
189	" 17	9	7	0
190	" 17	11	5	1
191	" 17	12	2	0
192	" 17	13	1	1
207	" 23	9	8	4
209	" 23	13	6	2
			80 63.5 %	46 36.5 %

Table VIII

Ovipositions under Mercury Arc - Filter No. 6

No.	Date	♀ No.	♂	♀
103	March 22	1	2	1
104	" 23	1	2	0
105	" 24	1	1	0
106	" 25	1	4	0
107	" 26	1	3	1
119	" 31	2	9	2
132	April 5	1	1	0
133	" 5	7	1	1
134	" 5	9	8	2
135	" 5	10	5	0
153	" 10	7	11	3
154	" 10	8	6	6
155	" 10	9	13	2
181	" 16	7	14	0
182	" 16	8	3	6
183	" 16	9	8	3
184	" 16	11	12	0
185	" 16	12	4	0
186	" 13	13	4	0
203	" 22	9	7	6
204	" 22	11	5	0
206	" 22	13	5	2
			128 78.6 %	35 21.4 %

Table IX

Ovipositions under Mercury Arc - Filter No. 9

No.	Date	♀ No.	♂	♀
111	March 28	2	10	5
122	April 2	5	11	4
123	" 2	6	19	6
163	" 13	7	3	1
164	" 13	8	2	1
165	" 13	9	1	0
166	" 13	11	3	1
168	" 13	13	5	3
195	" 19	12	2	0
196	" 19	13	3	0
212	" 25	9	11	1
213	" 25	13	11	3
			81 76.5 %	25 23.5 %

Table X

Ovipositions under Mercury Arc - Filter No. 1

No.	Date	♀ No.	♂	♀
113	March 29	2	8	7
124	April 3	1	1	0
125	" 3	7	16	3
126	" 3	8	11	7
142	" 8	2	2	6
143	" 8	7	6	1
144	" 8	8	4	4
145	" 8	9	4	3
169	" 14	7	2	1
170	" 14	8	2	2
171	" 14	9	1	0
172	" 14	11	8	0
173	" 14	12	2	0
174	" 14	13	8	1
197	" 20	9	12	1
198	" 20	11	7	1
			95	37
			72 %	28 %

Table XI

Ovipositions in Darkness

No.	Date	♀ No.	♂	♀
158	April 12	7	12	5
159	" 12	8	4	4
160	" 12	9	12	7
161	" 12	11	16	1
162	" 12	13	11	2
193-194	" 18	8 & 9	17	5
210	" 24	11	4	1
211	" 24	13	1	0
			77	25
			75.5 %	24.5 %

Arranged in the order in which they appear in the spectrum with the naked "mercury arc" group at one end and the "dark" group at the other, the various lights appear in the following order:

Mercury Arc	Mercury Arc - No. 13	Mercury Arc - No. 6
2500-7000 Angstrom units	3656 only. All Ultra Violet	No Ultra Violet 75 - 80% of white light
Mercury Arc - No. 9	Mercury Arc - No. 1	Dark
Approximately Pure Blue	Transmits Infra Red only	

The percentages of females secured are summarized graphically in this order. Figure 4.

Discussion

It is difficult to draw any inference as to the effect of light on the sex ratio from the figures obtained. The fact that the parasites not only oviposit in the dark but produce females in percentages comparable to those produced under most of the other light conditions, seems to indicate that presence or absence of light at time of oviposition is a matter of no importance with this species and that the slight variation of sex ratio under light of different qualities is not of sufficient extent to indicate any definite reaction.

Figure 4. Comparison of female production under various light frequencies.

Mercury Arc All effective rays	xxxxxxxxxxxxxxxxxxxxxxxxxxxx 28.5 % females
Mercury Arc - Filter No. 13	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx 36.5 % females
Mercury Arc - Filter No. 6	xxxxxxxxxxxxxxxxxxxxx 21.4 % females
Mercury Arc - Filter No. 9	xxxxxxxxxxxxxxxxxxxxxxx 23.5 % females
Mercury Arc - Filter No. 1	xxxxxxxxxxxxxxxxxxxxxxxxxxxx 28.0 % females
Dark No light used	xxxxxxxxxxxxxxxxxxxxxxxx 24.5 % females
Check Regular laboratory conditions	xxxxxxxxxxxxxxxxxxxxxxxxxxx 27.7 % females

EFFECT OF LIGHT DURING STORAGE AND FEEDING PERIODS

There seems to be little reason to assume that amount or kind of light during periods of storage and feeding of adults has any effect on the sex ratio of the resulting parasites. As indicated earlier, at times the sex ratio in the laboratory has been satisfactory. At such times, the parasites were handled in the same way as at other times when results were not so satisfactory. In addition to this evidence, there have been times when numbers of individual females were run separately to compare the sex ratios of their progeny. At such times the sex ratio has varied from 6.1 per cent females to 50 per cent females with the parasites stored and fed in identical fashion.

TEMPERATURE AND HUMIDITY AT WHICH ADULTS ARE KEPT

In handling parasitic insects in the laboratory, the temperature and humidity at which they are kept during their periods of inactivity are those at which they have been found to live longest. Chelonus and most other hymenopterous parasites remain in health longer if they are kept during periods of inactivity at from 40° to 50° F., with a high relative humidity. No data are available to indicate the influence on the sex ratio of higher temperatures during periods of inactivity. It was felt that, since the health and longevity of the parasites would be considerably shortened by any marked change in these conditions, little would be gained by experiments along these lines.

In so far as temperature at times of mating and oviposition is concerned, the parasites themselves limit this, there being little activity below 70°F., and so much above 80° F., that the life of the adults is materially shortened by higher temperatures. One would expect that, if the temperature and humidity were such as to shorten the life of the parasite, the tendency would be to further reduce the number of females. The practice, therefore, has been to maintain the temperature and humididy within the limits which promote the health and longevity of the parasites. Moreover, under these conditions, there has been a wide variation in the ratio of females, indicating that other factors are responsible for the variations observed.

Seasonal production of one sex or the other in greater or lesser abundance has been observed in many of the Social Hymenoptera (Bodenheimer) (57), and in aphids (Davidson) (58), (Marcovitch) (59). There is, however, in these forms, a reason to be found for this. In the Social Hymenoptera, at certain seasons an excess of males is necessary to insure mating, while at other seasons large numbers of males would prove a hazard to the continuance of the colony over periods of adversity. In the case of aphids, it has been definitely established, (Davidson) (58), that temperature affects the production of sexual forms, but, here too, this has a relationship to the perpetuation of the species. No data are available which indicate that, in parasitic forms, which have more than one generation a year, there is a seasonal fluctuation in sex ratio. Vance (7) states that in Northern Italy the sexes in Chelonus are about equal in both first and second generations.

There have been, however, observations which indicate that the same form occurring in different latitudes may have a consistently differing ratio of the sexes. Pelsener (11) states, "When one species has a geographic distribution extended in latitude, or when there are two closely related species (of the same genus), one northern and one southern, it can be shown, by numerous examples, that in the northern area the males diminish in number". There is both supporting and contradicting evidence in this connection. Holloway (60) for New Jersey reports that Macrocentrus ancylivorus maintains an almost constant ratio of 58 per cent females, while van Steenburgh (56) for Ontario reports an average percentage of females in the same species of 64.49. Morris (61) reports the opposite as the case in Exenterus abruptorius, in Hungary the percentage of females is 80, while in Sweden the sexes are approximately equal. In so far as the parasite dealt with in this paper is concerned, there is hardly sufficient evidence on which to base an opinion as to the effect of latitude. What data are available seem to indicate that the sex ratio may be adversely affected by the change of latitude. However, as in all cases, other factors must be considered in any definite conclusions which may be formed. The effect which may appear to be due to change in latitude may be due to host mortality or to other causes acting against one sex.

FOOD OF ADULTS

Two aspects of the effect of food on the activities of adult parasites require consideration, (1) presence or absence of food, (2) nature of food.

There is abundance of evidence in the literature to indicate that many, if not all, hymenopterous parasites do some feeding in the adult stage. Evidence is also present that this has a marked effect on the longevity and reproductive activities of the parasites. Doetn (62) states that, in Pteromalus puparum, males and females will not live more than a few days if not fed, but, if fed a mixture of honey and water, they will live for over two months. In this case, if females were not fed, oviposition activity would be practically eliminated by the shortness of life. Experience with Chelonus has been similar except that, for the most part, a light syrup of cane sugar has been used instead of honey-water. Doten (62) also indicates that individuals which have been without food for some time refuse to lay, but that, after they have had time to feed, oviposition will be resumed. Chelonus females which have been fully fed shortly before oviposition is sought are rather sluggish, and do not lay as well as those which have not been fed since the previous day. Apart from their health and longevity, there is no evidence to indicate that presence or absence of food has any effect on the sex ratio of the progeny. High and low percentages of females have been secured from both individuals and lots of females treated in an identical manner as to presence or absence of food.

Two types of food, carbohydrates and proteins, are known to be taken by adult hymenopterous parasites. In nature the carbohydrates probably consist of such substances as honey dew and nectar. In the laboratory such things as honey, honey-water, sugar, and sugar syrup are used for feeding. The protein food of parasites is usually obtained from the host at or subsequent to the time of attack. Fox (63) states that, in *Exeristes roborator*, females devour the tissues of the host, and that oviposition practically never occurs until such feeding has taken place. He further states that carbohydrate food will not bring about oviposition. *Trichogramma* adults have been observed by the writer to feed at the droplet of fluid which exudes from the egg as the ovipositor is withdrawn, but this has never been observed in *Chelonus*. It is probable, therefore, that *Chelonus* females feed only on carbohydrate substances, and that, provided the health of the parasites is not affected by continued lack of food, there is no effect on the sex ratio.

In the case of males, feeding is often necessary to get mating. Since, in the laboratory none but mated females are used for oviposition purposes, lack of food for males, and consequent lack of mating, are not factors in the sex ratio obtained.

CROWDING OF ADULTS

The conditions under which parasites are handled in the laboratory are highly artificial. One of the factors which contributes to this artificiality is that of crowding the parasites into cages. There are three times during which this crowding might affect the parasites in such a way as to upset the sex ratio; (1) during periods of inactivity, (2) at time of mating, (3) during oviposition.

As mentioned earlier, adults are stored during periods of inactivity at temperatures between 40° and 50° F. At these temperatures, activity is practically absent. Provided sufficient space is available for the insects to rest and the conditions are such as to promote longevity, no injurious effect on the sex ratio could be expected.

At the time of mating, it has been the custom to place a small number of females in a cage containing a large number of males. Males, on the whole, show considerably more interest in the mating activity than do females, and there is usually considerable competition among the males for the privilege of mating the females. It would seem that, under such conditions, mating might be hurried and the passage of seminal fluid incomplete. However, the adults remain in copula as long under these conditions as when conditions are less crowded or where pairs mate singly. The sex ratio of the progeny of adults mated under crowded conditions has never been noted to vary from that occurring where few adults are present at the time of mating.

In laboratory breeding of this species, two methods of securing ovipositions have been used. When breeding was first started, single females were placed in vials and allowed to oviposit in corn borer eggs approximately the number of times there were eggs in the egg mass. Two generations were run in this way, the first of which was from imported adults. The first generation gave 51.5 per cent females and the second 38.9 per cent females, both being handled in identical fashion as to the method of oviposition. In later work, females were allowed to oviposit over a period of several hours on a number of egg masses in a cage, as shown in Figure 5. Under such conditions there has been a wide variation in the sex ratio secured where the conditions as to oviposition were identical. A series of mated females was taken from stock and each run singly in a cage where crowding would be absolutely eliminated. The record of the progeny of these is given in Table XII.

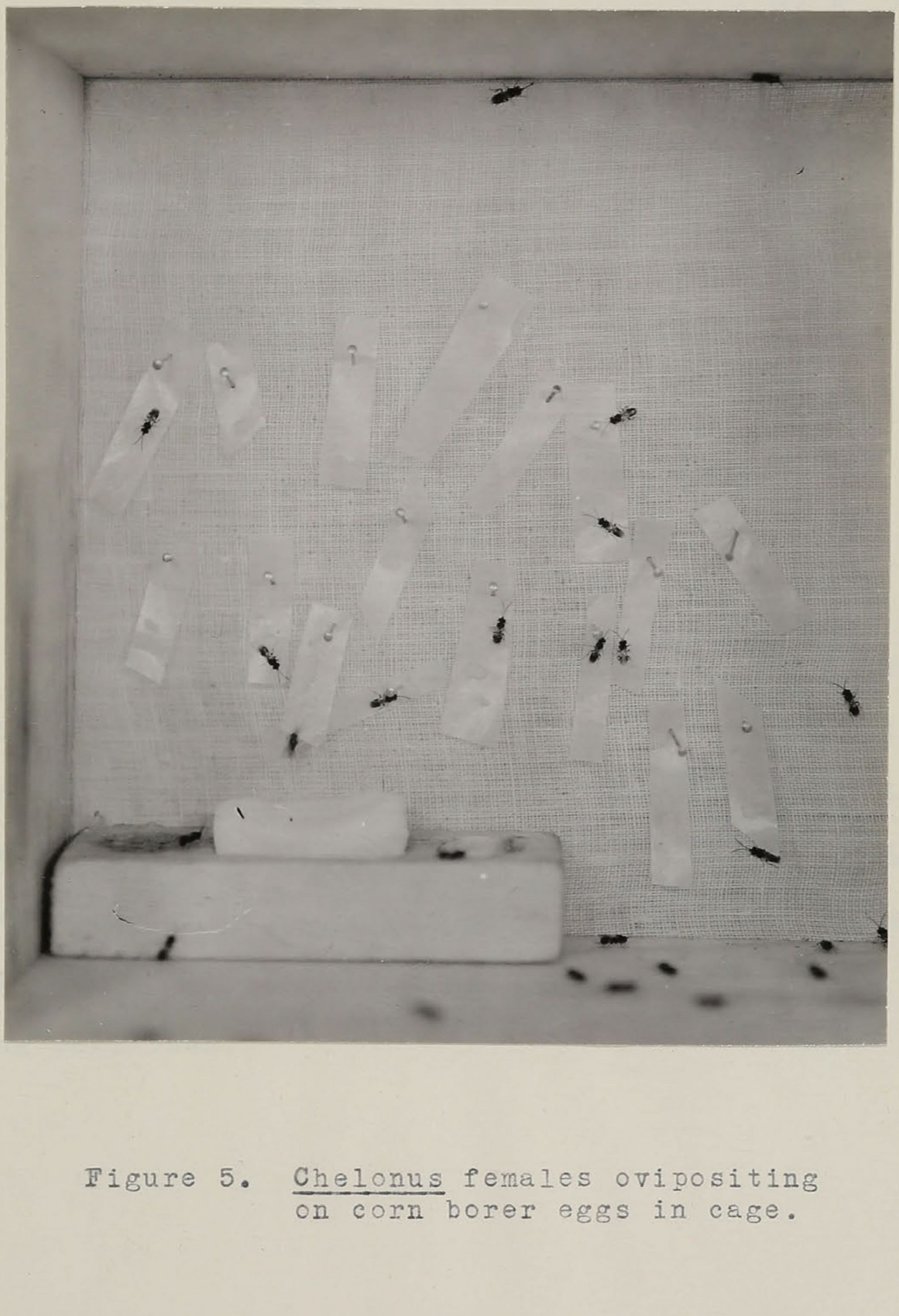

Figure 5. <u>Chelonus</u> females ovipositing on corn borer eggs in cage.

TABLE XII

Females Ovipositing Singly

Female No.	Offspring ♂	♀	% Females
20	61	4	6.1
21	34	16	32.0
22	48	15	23.8
23	34	13	27.8
24	20	2	8.8
25	60	11	15.4
26	29	4	12.1
27	26	20	43.5
29	29	26	47.3
30	17	5	22.7
32	19	19	50.0
33	23	3	11.6
34	27	5	15.6
35	35	13	27.0
36	51	35	40.7
38	47	14	22.9
	560	205	25.6

It is interesting to compare the average percentage of females in this group with what occurred in the regular stock during the same season. In 9,885 individuals the percentage of females was 27.7. These data indicate quite clearly that crowding occurring at time of oviposition has no effect on the sex ratio of the resulting parasites.

AGE OF HOST AT TIME OF PARASITISM

It was shown in some earlier work (Wishart and van Steenburgh) (1), that the age of the host eggs at time of parasitism had a considerable effect on the numbers of the host that were effectively parasitized, but no evidence was present to indicate that there was any effect on the sex ratio of the resulting parasites. There is a great deal of evidence in the literature concerning the effect of size of host on the sex of the offspring, but it is difficult to determine with certainty whether it is only size, or whether age of host is the determining factor. Van Steenburgh reports (56) that, in Macrocentrus ancylivorus, hosts parasitized while in the first stage produce ralatively more males than those parasitized when larger, but he expresses doubt as to whether age is the reason for this difference, since the mortality among the host larvae is greater in the earlier instars than in the later ones. Studies on the biology of Chelonus in the field by James (64) under the writer's direction, indicate that in the field there is a slight preference shown by the females for two-day old eggs. Parasites have been secured from hosts which were parasitized at times varying from immediately after the eggs were laid until just before eclosion of the host occurred. If any influence on sex is caused by age of host at time of attack, it is so slight as to be rather completely masked by other factors which could produce sufficient variability to explain the results secured.

RELATION BETWEEN DAILY EGG-LAY AND SEX RATIO

In the laboratory a plentiful supply of host material is presented to the parasites. In the field, the parasites undoubtedly use a lot of their time and energy in their search for the host. It seems natural to suppose that in the laboratory the parasites will lay a greater number of eggs per day than they do in nature. The question arises as to what effect this may have on the sex ratio.

As indicated elsewhere, Chelonus, when ovipositing in the eggs of the corn borer, shows little discrimination between eggs which have already been parasitized and those which have not. In addition, it has been demonstrated by repeated dissections that females may puncture a host egg and appear to deposit an egg without doing so. This makes it impossible to observe, without destroying the host and parasite, how many eggs a female Chelonus lays in a day, and precludes any possibility of determining the effect of the number of eggs laid daily on the sex ratio in this species. It was thought, however, that some information in this connection could be secured with Microbracon brevicornis, which deposits its eggs externally. For this purpose, the egg-lay of twenty-five females of this species is presented in Table XIII. In figure 6. the average daily egg-lay is plotted against the percentage of females for each female. No co-relation appears to exist between average number of eggs laid and the percentage of females produced.

Table XIII

Female No.	No. Days	Total Eggs	Average Daily Eggs	♂	♀	% ♀
1	9	222	24.66	63	112	64.00
2	9	180	20.00	39	105	72.91
3	9	199	21.11	91	63	40.90
4	4	88	22.00	43	10	18.86
5	11	253	23.00	98	52	34.66
6	9	213	23.66	98	32	24.61
7	7	215	30.71	42	80	65.57
8	9	124	13.77	33	15	31.25
9	6	95	15.83	18	64	78.04
10	11	243	22.10	46	136	74.72
11	10	236	23.60	54	96	64.00
12	8	168	21.00	69	51	42.50
13	8	160	20.00	31	54	63.53
14	8	116	13.75	38	23	37.70
15	8	145	18.12	36	37	50.68
16	10	181	18.10	27	47	63.51
17	9	129	19.33	26	30	53.37
18	8	154	19.25	82	21	20.38
19	3	22	7.33	5	5	50.00
20	9	156	17.33	21	71	77.17
21	7	145	20.71	81	44	35.20
22	7	37	5.29	11	18	62.06
23	20	160	8.08	23	51	68.71
24	17	173	10.17	55	39	41.48
25	17	165	9.70	36	41	53.24

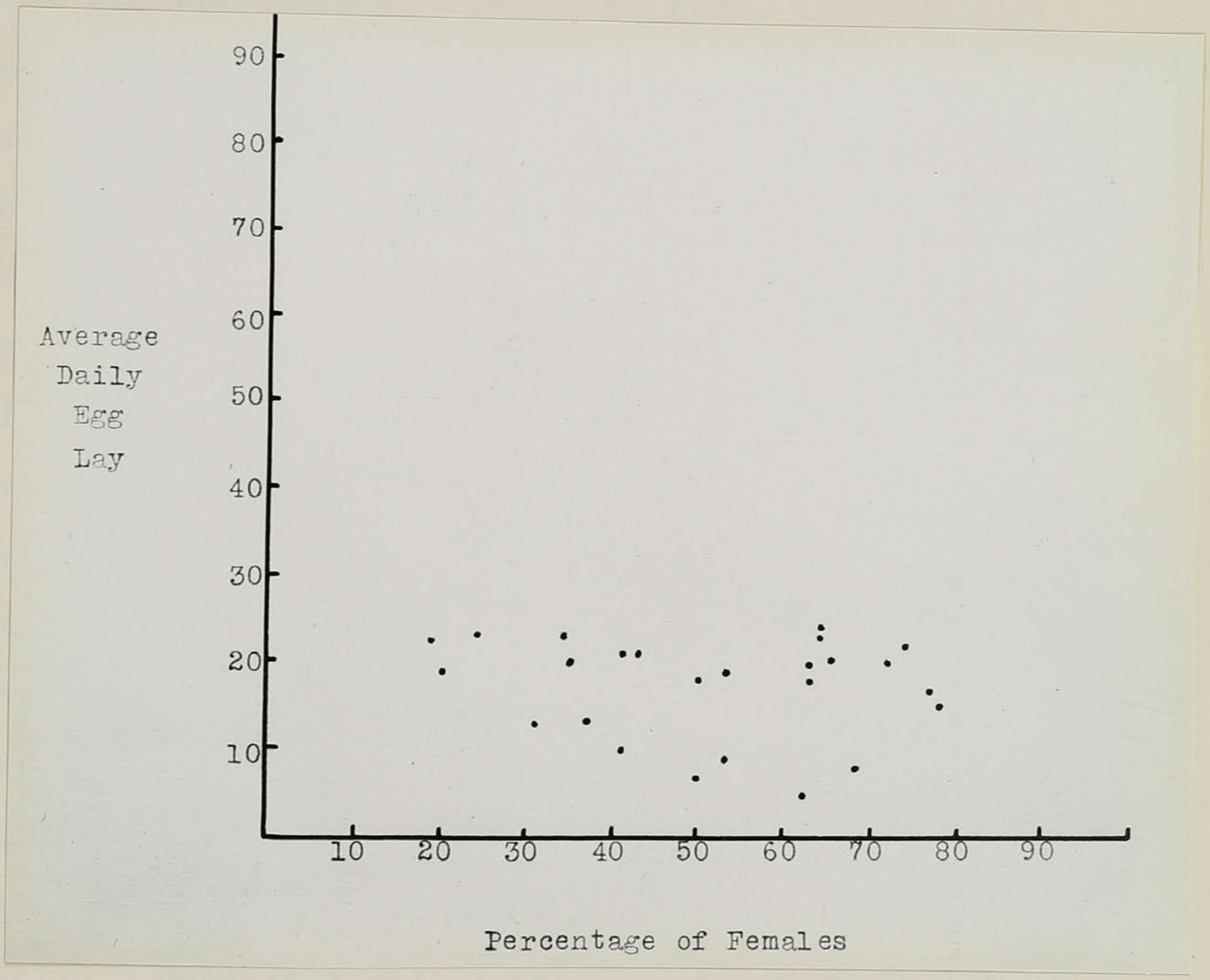

Figure 6. Average daily egg lay and female production.

SELECTIVE ELIMINATION OF ONE SEX DURING LARVAL DEVELOPMENT

When rearing Chelonus annulipes on corn borer larvae, there has always been a considerable mortality among the host larvae. It is, in fact, difficult to rear through high percentages of unparasitized larvae. This mortality varies from time to time. It is probable, therefore, that the host larvae, during the period in which they are carrying the parasite larvae, are very sensitive to any additional adverse conditions. The opinion is held in some quarters, (Goldschmidt) (25), that host individuals bearing one sex may suffer more mortality due to the greater demands for food which this sex may exhibit. If this were the case in Chelonus, and potentially-female parasites made greater demands on the host than potentially-male parasites, a co-relation between the extent of the mortality and the proportion of males and females should appear.

Some uncertainty was felt as to what data would be of use in this respect. Individual females appeared to vary in their female-producing capacities. The question arose as to whether an analysis of the day-to-day production of individual females, or a study of the mean survival and mean female production of a very large group, would be more significant. The latter method was chosen, and the production of fifty females used for this purpose.

Corn borer larvae, parasitized by Chelonus, are reared en masse in earthenware saucers until the host larvae are about eight days old, at which time the larvae are isolated in vials. While the larvae are in the saucers no record of mortality is kept, but, after isolation, records are kept of the fate of each larva. The lack of data in the early part of the parasite and host life is unlikely to be of much significance since, up until this time, the parasites are still in the first stage and their demands for food are so small as to create very little, if any, differential demands on the host. In computing survival, all larvae, which formed cocoons or were discarded as unparasitized at the time cocoons were formed, were considered as surviving. The percentage survival was found by taking this figure with the number of larvae isolated into vials. Each day's production for each female was worked up separately, and then the mean survival and the mean percentage of females were secured for each parent female from these figures. Table XIV shows the number of days each female was used, the mean survival, and the mean percentage of females. In Figure 7. the mean survival figures are plotted against the mean percentage of females for each parent female.

Reference to the figures and the graph fail to show any trend to indicate that heavy mortality among the

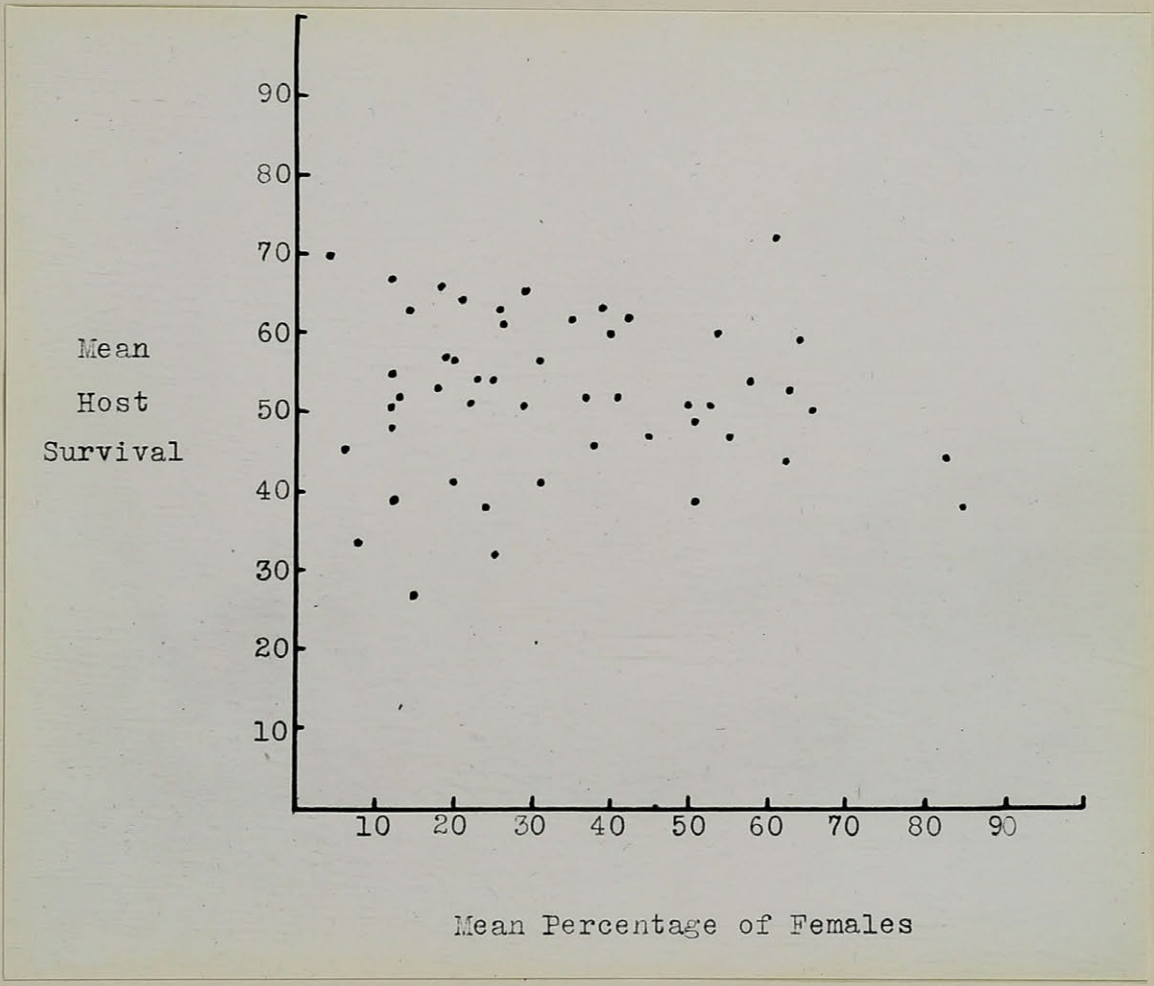

Figure 7. Host survival and female production.

host larvae is co-related with a greater or lesser production of females.

Table XIV

Survival and Production of Females

Female No.	No. Days	Mean Survival	Mean % ♀
20	9	70	4
21	6	63	39
22	8	66	18
23	5	57	20
24	3	52	13
25	7	67	12
26	8	55	12
27	6	49	51
29	10	51	50
30	5	46	6
32	5	54	58
33	6	57	19
34	7	62	35
35	6	53	18
36	11	52	37
38	8	64	21
50	9	62	42
51	8	63	26
52	5	59	64
53	9	57	31
54	11	51	29
57	13	60	40
59	3	72	61
56	18	53	63
55	17	63	14
66	5	46	38
67	2	34	8
68	2	48	12
69	2	51	12
70	16	50	22
72	10	65	29
73	9	50	66
74	8	39	12
75	6	54	23
76	3	27	15
78	6	52	41
79	2	61	26
82	6	32	25
83	4	54	25
86	9	47	45
87	10	39	51
88	4	41	20
90	5	38	24
91	6	47	55
92	7	41	31
94	7	51	53
96	8	38	85
97	10	60	54
98	5	44	63
99	2	44	83

SELECTIVE ELIMINATION BY MORE RAPID DEVELOPMENT OF ONE SEX

As indicated earlier, Chelonus annulipes, when ovipositing in the eggs of the corn borer, frequently deposits more than one egg in a single host egg. In approximately forty thousand individuals of this species, which have been reared, in one case only has there been more than one parasite emerge from a single host. It is evident, therefore, that where two or more eggs are deposited in a single host, there is elimination of all but one parasite. It seems logical to suppose that the larva which hatches first is the stronger and, therefore, eliminates any which hatch later. This elimination takes place very shortly after hatching. It was shown earlier that there is as wide a spread in hatching time in eggs laid by virgin females as in those laid by mated females. It would appear, therefore, that elimination, due to later hatching of potentially female-producing eggs, is not responsible for any selective elimination at this point.

It may be well here to indicate what proportion of the parasitized eggs receive more than one egg. Examination was made of 299 laboratory parasitized eggs with the following result:

250 contained 1 parasite egg
39 " 2 " eggs
8 " 3 " "
2 " 4 " "

Since no definite information was secured on the proportion of fertilized eggs which were laid, it was impossible to compute what change in the sex ratio was brought about by selective elimination of females due to more than one parasite egg being laid in one host. It is evident, however, that even if all females were eliminated in the superparasitized eggs, the difference would not be sufficient to explain the difference in sex ratio in the laboratory and in the field where the parasite occurs naturally. Moreover, this occurrence is also present in the field, though to a lesser extent. James (64) reports that, in 48 eggs found parasitized in the field, three had two parasite eggs in them.

Some further evidence may be added to the above. In 1937, Ephestia was used as host in the rearing of this parasite. Dissection of one hundred eggs of this host, exposed to Chelonus, failed to reveal one case in which more than one egg was laid in one host. Even when a very small number of host eggs were presented to a large number of parasites, the same result was secured. In the rearing of fourteen thousand Chelonus on this host, there were 35.8 per cent females, as compared with the previous year, when 33.8 per cent females were secured from corn borer (see table II).

EFFECT OF TEMPERATURE AT WHICH HOST LARVAE ARE REARED

The desirability of investigating the effect on the sex ratio of _Chelonus_ of the temperature at which the parasitized host larvae are reared has long been appreciated, but two factors have, until recently, prevented this from being done. Until the spring of 1937 the practice had been to rear _Chelonus_ on its normal host, _Pyrausta nubilalis_. The latter had been reared in the laboratory on such succulent foods as string beans, curled dock, (_Rumex crispus_), and mangels. Long experience has shown that to get satisfactory numbers of the host through they must be reared at temperatures of from 80° to 85° F. Lower temperatures prolong the time of rearing, and thus increase the hazards, making the mortality much higher. In the second place, the strain of the corn borer with which we have been working is known as the one-generation strain, having but one generation per year. It is very easily thrown into diapause, and, to prevent this, great care must be taken to maintain the growing larvae at constantly high temperatures. Even with such precautions, a relatively small proportion of unparasitized laboratory reared larvae will form pupae. This tendency to diapause is important in its effect on _Chelonus_ which appears to have no definite tendency of its own, but seems to respond to the reaction of the host in this respect. That is, if the host has a tendency to go into diapause when the temperature is lowered, the parasite will do likewise. The matter is so critical in the case of the corn borer and *Chelonus* that in the writer's work there has always been a very small percentage of *Chelonus* go into diapause. W. A. Baker (65), reports that his attempts to rear *Chelonus* on corn borer were unsuccessful from a practical standpoint, due to the high proportion of the parasite larvae which went into diapause. It will be evident from the above that experiments with varying temperatures at the time of rearing of the parasitized host larvae were impractical as long as the corn borer was used as the host.

Early in 1937 following the advice of W. A. Baker (66) breeding on *Ephestia kuehniella* Zell. as host was started. *Chelonus* attacks the eggs of this insect much less readily than those of the corn borer, and due to its smaller size the parasites themselves are smaller. Otherwise, however, *Ephestia* has proved to be a very satisfactory host for large scale laboratory breeding. Also, since it shows no diapause tendency whatever, it made possible the study of the effect of temperature at which the parasitized larvae are reared on the sex ratio of *Chelonus*. At the time of writing the study is incomplete, but it is felt that sufficient data are available to be of some significance.

The first experiment was designed to determine the effect of temperature on the sex ratio of the present generation. *Ephestia* eggs stuck to cards were presented for parasitism after which each card was cut into four approximately equal sections. The eggs on each section were counted, and placed in a jar with one gram of whole wheat meal for each egg. The jars were then placed in four incubators, operating at the following temperatures: incubator A - 90° F., incubator B - 80° F., incubator C - 70° F., incubator D - 60° F. A total of twenty-five lots was thus treated. In the material incubated at 60° F., the temperature apparently was below the threshold of development for *Chelonus*, as none of the parasites advanced beyond the first stage. The data for the other three temperatures are given in Table XV. Although the percentages of parasitism secured were quite low, and the numbers in the 70° F. class lower than in the others, the number of females was greater than in those incubated at 80° F. or 90° F., and the percentage of females was considerably greater. If the data are sufficient to be significant, they would appear to indicate that there was some selective elimination of females at the higher temperatures.

The second experiment was designed to test the effect on the sex ratio when the parents were reared at high or low temperatures. For this purpose males and females reared at 90° F. were mated, and males and females reared at 70° F. were mated. Eggs were presented for parasitism to each group at room temperature (72°F.), and

TABLE XV

Effect of Temperature at which Host Larvae are Reared on Sex Ratio

A 90°F.				B 80°F.				C 70°F.			
Eggs	♂	♀	% ♀	Eggs	♂	♀	% ♀	Eggs	♂	♀	% ♀
57		1	100	39	6	1	14	30			0
60	4		0	45	1		0	55			0
79	16		0	83	9		0	72	14		0
62	14	1	6	50	11		0	68	9		0
70	12	1	7	72	14	7	33	74	7	5	41
112	4		0	104	3		0	84	4		0
81	8	2	20	89	3	5	62	90	7	6	46
81	6		0	103	13	2	13	80	3	1	25
111	28	12	30	103	23	4	14	97	18	9	33
103	26		0	117	38	2	5	123	19		0
160	26	4	13	162	28	2	6	106	6	1	14
140	10	1	9	143	19	0	0	152	3	1	25
162	1		0	148				176			
103	4		0	112	5			115	3		
107	13		0	93	16	3	15	114	6	1	14
130	7	0	0	78	5	1	16	142	8	5	38
116	1		0	110	2	1	33	123	3	1	25
98	6	1	14	108	2		0	118			
99	3		0	96	8	2	20	100	2	1	33
198	3	2	40	152	11	1	8	170	12	2	14
143	4		0	154	8	2	20	153	6	2	25
110	1		0	132	7		0	129			
163	2		0	126	1	2	66	160	4	1	20
105	4		0	114	7		0	104	3		0
114	5		0	124	6		0	123	2	1	33
	208	25	10.7		246	35	12.4		139	37	21.0

Table XVI

Effect of Temperature at which Parents are Reared on Sex Ratio

Parents Reared at 90°F.				Parents Reared at 70°F.		
Progeny Reared at 80°F.				Progeny Reared at 80°F.		
♂	♀	% ♀		♂	♀	% ♀
29	10	25		3	6	66
32	9	20				
50	11	18				
36	20	35				
36	11	23				
4	5	55				
29	0	0		5	34	87
23	0	0				
29	0	0		11	15	57
29	0	0				
21	0	0		9	12	57
58	0	0		7	1	12
				3	7	70
376	66	14.9		38	75	66.3

the parasitized material was all reared at 80° F. The results are presented in Table XVI.

The data indicate that individuals reared during their larval life at high temperatures are incapable of producing as high a percentage of females as those reared at low temperatures. Since this species is arrhenotokous, producing males without fertilization, and, since males were produced quite freely from the material reared at 90° F., it would appear that the deleterious effect operated against the males. Young and Plough (1926) (37) found that in <u>Drosophila</u> high temperatures have a differential effect, such that males may be rendered completely sterile at a point at which females are still completely fertile, and that high temperatures seem to reduce the motility of the sperms. A partial sterility of the males may have occurred in the males reared at 90° F or, as suggested by Young and Plough, the results may have been produced by a lack of motility in the sperms.

More extensive work would be required to definitely settle the matter, but the data are the most convincing so far obtained in this study.

CHANGE OF SEX

Undoubtedly the chief factor in determining the sex of most individuals belonging to the Hymenoptera is the chromosome composition: if they have the haploid number for the species, they are likely to be male, if they have the diploid number they are likely to be female. However, so much evidence has been accruing to prove that sex is not irrevocably fixed at time of fertilization that the matter of change of sex should be given some consideration, where the sex ratio deviates greatly from the normal. Unfortunately, some of the workers in other forms who have produced change in sex, (Holdaway) (23), (Christie) (24), have not indicated any evidence as to how the chromosome constitution in the changed forms compares with the normal forms, or as to whether or not those whose sex has been apparently changed, reproduce normally. Whiting (39), working with Habrobracon, has produced what were very evidently diploid males. In these, however, there was a high degree of sterility. Some of the workers who have noted the preponderance of male parasites from small hosts and female parasites from large hosts have assumed that it was the quantity of food which determined the sex of the parasite. The evidence that in many species size of host is related to sex of the resulting parasites is such that we are forced to accept one of two hypotheses. Brunson (51), as indicated earlier, has shown that in

Tiphia popilliavora eggs moved from small hosts to large still give males, and those laid on large and moved to small hosts give mostly females. For this case and probably for other similar ones, we must reject the hypothesis that quantity of food influences the sex of the parasites, and accept the hypothesis that the females lay unfertilized or haploid eggs on small hosts, and fertilized or diploid eggs on large hosts.

In the parasite with which we are dealing, what criteria should be used in determining whether or not change of sex is responsible or partly responsible for the deviation of the laboratory sex ratio from the normal sex ratio? The first which should be considered is perhaps the presence or absence of diploid males. As indicated elsewhere in this thesis, no heritable mutations have been observed in this species, and none were produced by the x-rays in the quantities administered. It was, therefore, impossible to determine if any males had maternal characters and were, therefore, diploid. Had diploid males been present in large numbers, there would have been considerable evidence of sterility apparent. Such did not occur, fertility being the rule where mating took place. It is very probable also that if sex reversal were taking place, due to food, or some other factor, intersexes would have appeared in considerable numbers. In the rearing of many thousands of individuals, all of which were classified as to sex, no abberant forms were found. It would seem, therefore, that there is no reason to believe that change of sex, due to environmental factors, has been responsible for the poor ratio of female parasites secured in the laboratory.

HEREDITY

Incidental to the experiments with the effect of light on Chelonus, some data were obtained which indicated that there was a wide variation in the proportions of the sexes produced by different individual females. These data were such as to suggest the necessity of investigating the possibility that the faculty of producing large numbers of female offspring might be hereditary. Reference to Table XII, page 52, indicates the variation in sex ratio which may occur in the progeny of a group of females handled under identical conditions.

Some evidence along this line was also secured with Microbracon brevicornis. Two females were tested as to their female-producing capabilities, and their female offspring were also tested in the same manner. In Table XVII the production of the daughters of these two females is given. It seems more than a coincidence that almost all the daughters of female No. 2 were low in female production, and that almost all the daughters of No. 5 were high in female production.

It was realized at the outset that there were certain difficulties in the way of getting any satisfactory data in this connection with Chelonus. The character in question is invisible and can only be measured by breeding experiments. When a female has been tested as to her capabilities, it is too late to make whatever matings would be desirable. The desirability of having some visible mutation was evident, and, in view of the apparent ease with which these have been produced in *Drosophila*, *Habrobracon*, and *Galleria*, it seemed wise to attempt to obtain some in *Chelonus*. Through the co-operation of the radiologist at the local hospital, arrangements were made to expose *Chelonus* in the proper stage to x-rays for periods up to thirty minutes. No mutations appeared and, due to local conditions, longer exposures could not be secured.

Table XVII
Inheritance of high female production
Microbracon brevicornis

Female No.	No Days	♂	♀	Total	% ♀	Host	Source of ♀
2	3	9	8	17	47.06	*P. nubilalis*	stock
13	4	32	23	55	41.81	"	2
15	13	140	71	211	33.65	"	2
16	14	196	7	203	3.57	"	2
17	11	147	60	207	28.98	"	2
18	11	105	17	122	13.93	"	2
20	6	24	32	56	57.14	"	2
		644	210	858	24.4		
5	10	30	67	97	68.45	*P. nubilalis*	stock
21	10	63	112	175	64.00	"	5
22	9	39	105	144	72.92	"	5
23	9	91	63	154	40.99	"	5
24	3	43	10	53	18.81	"	5
28	6	33	15	48	31.25	"	5
29	5	18	64	82	78.05	"	5
30	11	46	136	182	74.72	"	5
31	9	54	96	150	64.00	"	5
32	8	69	51	120	42.50	"	5
33	8	31	54	85	62.22	"	5
34	6	38	23	61	37.70	"	5
46	16	23	51	74	68.92	*Ephestia*	5
47	14	55	39	94	41.49	"	5
48	15	36	41	77	53.24	"	5
		639	860	1499	57.3		

From the females whose female production was known (Table XII), two which gave the highest ratio of females were used for foundation stock, and selection for high female production was carried out through several generations. Both inbreeding and outcrossing were used. The results were not completely consistent throughout, but it is felt that they may have a considerable degree of significance. Table XVIII gives an analysis of some of these data. The parent generation (P 1) consisted of the two females mentioned above. The F 1 generation was an outcross from these, the F 2 generation consisted of both inbreeds and outcrosses of the progeny of the F 1 generation and so throughout. Where there was inbreeding, the degree of this is indicated. It will be noted that in the F 2 generation the inbreeds produced relatively more females than the outcrosses, while in the F 3 and F 4 generations, when inbreeding was carried farther, the inbreeds showed lower percentages of females than the outcrosses. A typical pedigree chart is shown in Figure 8. The percentages of females are indicated and also the number of progeny (50 % ♀ : 38 P = 19 ♂ and 19 ♀). This shows more clearly the effects of inbreeding and outcrossing. It is evident that inbreeding may increase the proportion of females at first, but, if continued, will not only reduce the proportion of the females, but also the number of progeny, and, in addition, a certain amount of sterility occurs.

Table XVIII
Selection for high female production

Generation	No. ♀ used	Total Progeny	% ♀ in total progeny	Inbreeding		Outcrossing	
				% of ♀ inbred	% of ♀ in progeny from inbred ♀	% of ♀ Outcrossed	% of ♀ in progeny from out-crossed ♀
P 1	2	93	48	0		100	48
F 1	7	414	39	0		100	39
F 2	15	318	34	33	36	66	33
F 3	14	275	44	35	37	65	48
F 4	16	301	25	50	20	50	30
F 5	10	170	32	100	32	0	
F 6	5	76	26	60	30	40	20

Typical Pedigree Chart -- Showing Effect of Inbreeding and Outcrossing

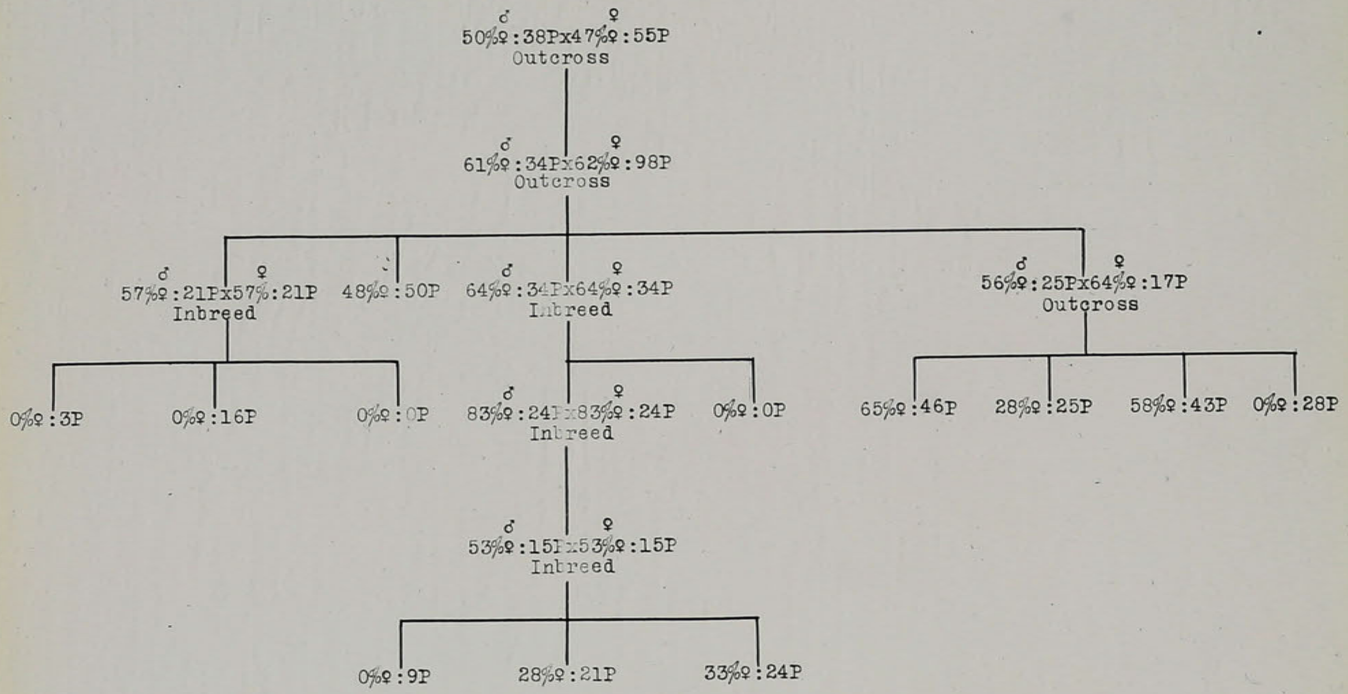

Figure 8.

CONCLUSION

The experiments carried out indicate that many of the environmental factors which it was felt were important in their relation to sex ratio in Chelonus have no significance. It appears also that the reduced number of females secured in the laboratory is the result of lack of fertilization of a sufficient number of the eggs laid by mated females and not of any selective elimination. The practice of rearing the parasitized host larvae at high temperatures is shown to reduce the number of females in the offspring. In the laboratory many successive generations of this species have been reared without any new introductions of stock. It is very probable that considerable inbreeding has occurred. This is also shown to affect the sex ratio adversely. It is the writer's opinion that these two factors have sufficient significance to explain the low number of females obtained in the laboratory.

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