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LENGTH OF LIFE OF THE SEXES IN *NASONIA VITRIPENNIS* (WALKER) (HYMENOPTERA, PTEROMALIDAE) UNDER CONDITIONS OF STARVATION

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INTRODUCTION

Grosch (1948) observed that in a starved population of *Habrobracon juglandis* the mean length of life of the male was significantly shorter than that of the female although the fat cells in the males were larger than those in the females. Georgiana (1950) found that the females outlived the males in honey-fed cultures of the same insect. Hase (1922) demonstrated that there is a difference in the chemical composition of the faeces of sexes in braconids. This is probably related to a difference in the feeding habits of the sexes. If the metabolic processes of the sexes are alike in starved braconids the female, because she lives longer under starvation than the male, would appear to have an additional store of food other than the fat body which is the only major store in the male. Flanders (1942) stated that when a hymenopterous parasitoid is unable to find a host egg production continues, though at a lower rate, and the mature eggs in its ovariole undergo a process of resorption. Bearing this in mind Grosch (1950) put forward the hypothesis that the resorption of eggs accounts for the longer life of the female, by supplying it with a source of reserve nutrients which is not available to the male insect.

In *Habrobracon juglandis* there is an egg reservoir situated at the point of union of the ovarioles of each ovary (Bender, 1943). It is possible that eggs are stored in this reservoir if hosts are not available. In the present study it was necessary to repeat on *Nasonia vitripennis* the work of Grosch because this insect does not have a reservoir, so the timing of egg resorption may be different, and egg resorption may begin sooner in insects which lack a reservoir than in those which have one (King, 1959). When the number of eggs increases in the ovarioles, however, because of lack of host puparia and because the female will not deposit eggs other than in a host puparium, a slight overlap between adjoining eggs takes place which to some extent may be a substitute for the reservoir.

Nasonia vitripennis is parasitic on the pupae but within the puparium of various muscoid flies. In laboratory cultures the female *N. vitripennis* was fed upon honey and host juices while the male had only honey. The male, having no ovipositor, is unable to drill into the host puparium and is thus unable to make a feeding tube and suck the host juices as does the female. It is possible that the male could bite its way into the host puparium and gain access to the body fluids, but this has not been observed.

Cousin (1933) stated that the length of life of adults of *N.* (*Mormoniella*) *vitripennis*

depended on nutrient. The length of life of the male was much shorter than that of the female. At a laboratory temperature of 18–20° C. the length of life of the males was 5–7 days whilst the life of the females was at least 20–25 days. Roubaud (1917) stated that the maximum age of the female of this species was 45 days. Presumably the parasites were fed in these experiments.

In the present study it is first established that under conditions of constant temperature and humidity the females of starved *N. vitripennis* live longer than the males. Then an attempt has been made to relate feeding methods to the condition of the ovaries as was done by Edwards (1954). Evidence is then given bearing on the hypothesis that the metabolism of the female differs from that of the male (Grosch, 1950). If the metabolic rate of the male is higher than that of the female it would account for her longer life under starvation conditions without the necessity for an extra food store such as resorbed eggs might provide. Grosch discussed this possibility but did not test it experimentally.

Starved specimens of both sexes of *Nasonia vitripennis* were placed in uniformly sized 3 in. × 1 in. tubes 1 day after emerging from the *Lucilia sericata* puparia in which they had developed. Each tube was closed with a cork, through the centre of which a hole was bored and covered with nylon gauze to allow air to enter the tube. Each tube contained 28 specimens in the ratio of approximately 1 male to 3 females. Thus the contents of each tube were similarly subjected to possible effects of crowding, including the searching for and courting of the females by the males, with any attendant increase in metabolic rate.

Every 6 hr. the tubes were examined and any dead specimens removed, their sex, width of head and age at death being noted. The width of the head was taken as a criterion of the size of the insect because this does not vary markedly with the nutritional state as other external measurements may do. The tubes were stored at a constant temperature of 25° C. and relative humidities of 100 and 65%.

RESULTS

As shown in Figs. 1–4, and the statistical analysis of these results in Table 1, there is a significant difference in length of life between the males and females at both 65 and 100% relative humidities. At R.H. 65% the rate of water loss may have caused the smaller males to be desiccated and thus die first, but this does not apply in the experiment conducted at 100% R.H. Another consideration is that the males die sooner than females of the same size, so the mean difference in size of the sexes cannot account for the differential mortality.

A second series of experiments was performed similar to the first except that unmated specimens were used. Parasitized puparia of *Lucilia sericata* were opened and the *Nasonia vitripennis* were removed 1 day before they were due to emerge, which means that in most instances the parasite had either just emerged from its pupa or was on the point of doing so. Each parasite was then kept in a separate tube. In such conditions it can be assumed that the parasites were unmated (Barrass, 1960). The tubes with the parasites in them were examined every 6 hr. and when the parasites died their head width, sex and age were noted. The results are presented in Figs. 3 and 4 and a statistical analysis in Table 1. The difference in length of life of the sexes

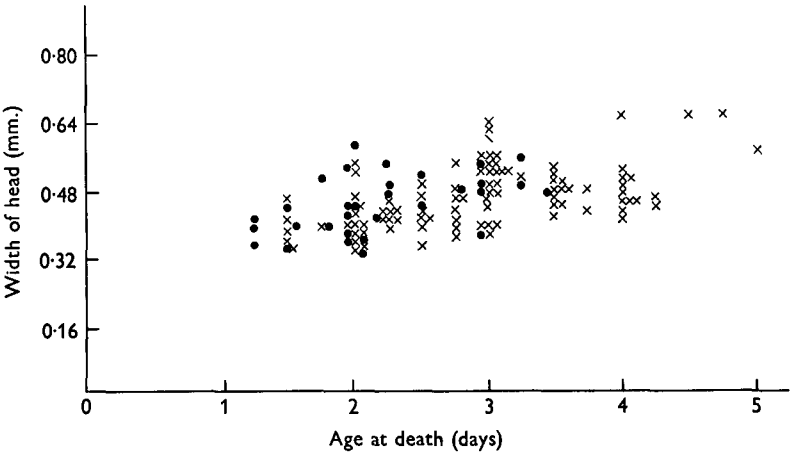


Fig. 1. Comparison of the size and age at death of the sexes. Specimens were mated and kept at R.H. 65% and temperature of 25° C. ×, Female; •, male.

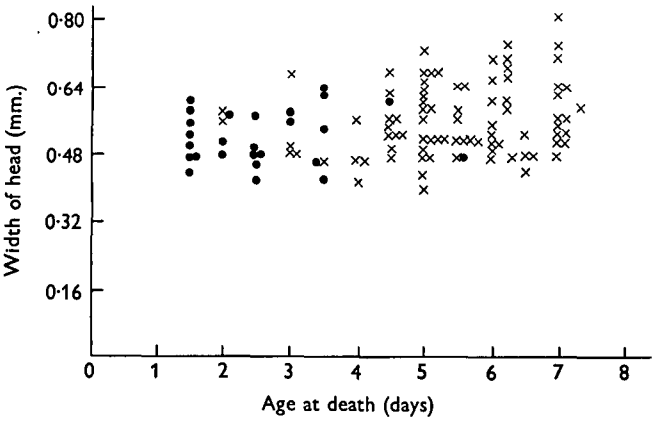


Fig. 2. Comparison of the size and age at death of the sexes. Specimens were mated and kept at R.H. 100% and temperature of 25° C. ×, Female; •, male.

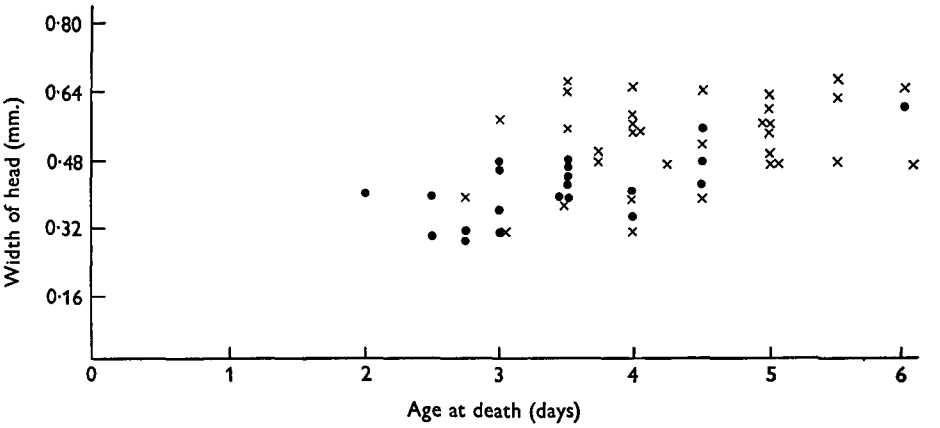


Fig. 3. Comparison of the size and age at death of the sexes. Specimens were unmated and kept at R.H. 65% and temperature of 25° C. ×, Female; •, male.

at relative humidities of 65 and 100% was again significant ($P < 0.01$) in both cases. The figures show, in conjunction with experiment I, that the incidence of mating does not affect the tendency for males to die before females of the same size.

There are two possible causes of the difference in longevity of the sexes. Either (*a*) the female has a different nutriment basis for her metabolism, which may give her a lower metabolic rate, or (*b*) she possesses an extra source of food which can be used during starvation.

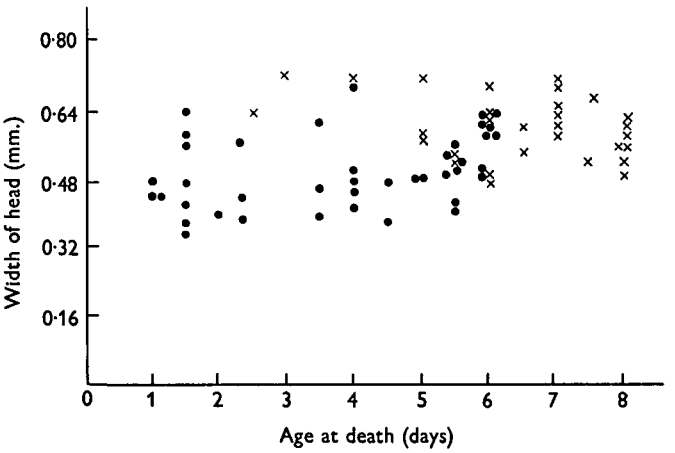


Fig. 4. Comparison of the size and age at death of the sexes. Specimens were unmated and kept at R.H. 100% and temperature of 25° C. ×, Female; •, male.

Table 1. *Analysis of results*

Treatment		Age at death in days: Males Mean	Males Standard error	Males No.	Females Mean	Females Standard error	Females No.	Diff. of mean	*t*	*P*
R.H. 65%	Unmated	3·525	0·70	20	4·416	0·15	36	0·89	7·12	< 0·001
R.H. 100%	Unmated	3·821	0·267	42	6·435	0·425	31	2·614	5·196	< 0·001
R.H. 65%	Mated	2·287	0·110	33	3·524	0·174	103	1·255	6·25	< 0·001
R.H. 100%	Mated	3·274	0·19391	73	5·74	0·1257	123	2·466	10·733	< 0·001

A. Investigation of the metabolic rates of the sexes

Two methods were used in this investigation. First, that described by Umbreit, Burris & Stauffer (1949), using Brodie's fluid in Warburg manometers. The flasks of the apparatus were kept in a constant temperature water bath at 25° C. They were not agitated because this might have produced abnormal movements in the insects. Five separate flasks were used containing:

(1) no insects;

(2) 15 females of *N. vitripennis*;

(3) 15 females with 10 ml. KOH in the well of the flask, together with a piece of filter paper to increase the surface of the KOH and thus its ability to absorb CO_2;

(4) 15 males of *N. vitripennis*;

(5) 15 males with 10 ml. KOH in the well of the flask.

In this series of experiments the difference in size between the sexes was eliminated by selecting large males and small females, so that the volumes of respiring tissue were similar for both sexes. The number used was the optimum necessary to produce a marked change in the manometer reading, whilst avoiding mortality before completion of the experiments. The apparatus was allowed to run for 2 hr. to allow it to reach equilibrium before any manometer readings were taken. These were then taken at 15 min. intervals for 3 hr. The rates of O_2 uptake and CO_2 output are similar in the two sexes (Figs. 5 and 6). It would therefore appear that the males have the same

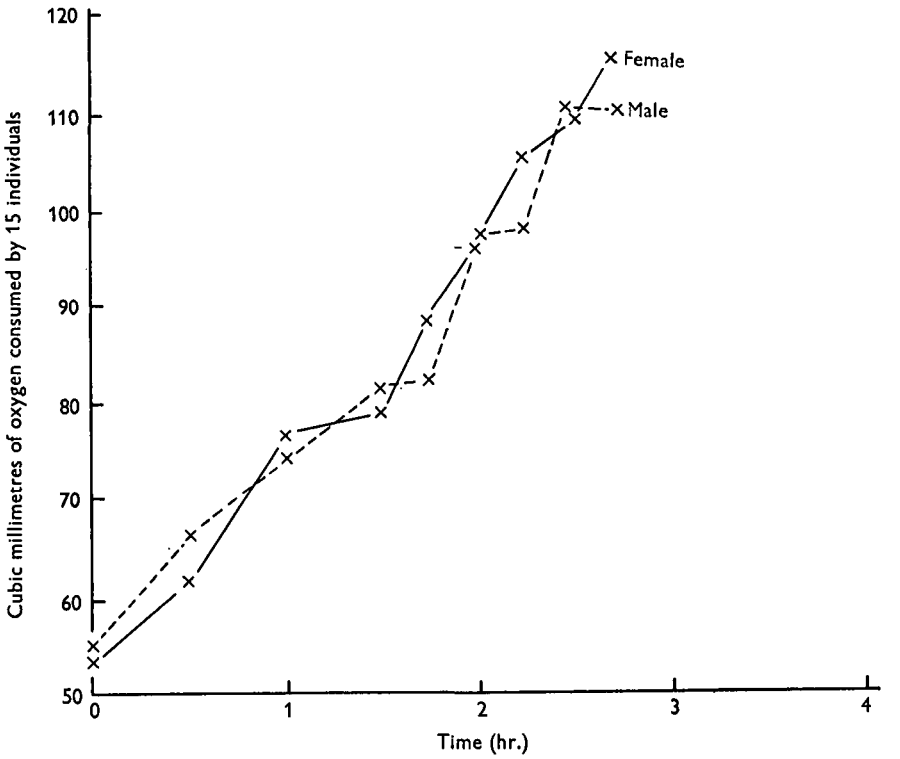


Fig. 5. Comparison of the uptake of oxygen by the sexes of *N. vitripennis*.

metabolic rate per unit volume of metabolizing tissue as the females. This method, however, is difficult to use over 24 hr. periods and continuous recordings over this period are essential since the metabolic rates of the sexes may exhibit different rhythms over this time.

The second method used to investigate the metabolic rates of the sexes was the system for continuous respirometry developed by Macfadyen (1961), which enables readings to be obtained continuously over several days. Single specimens of *N. vitripennis* were used in each cell of the apparatus. After emerging, some of the parasites were allowed access to host puparia on which the females feed before being put into the cells of the apparatus, in which they had no nutrient. Others were immediately placed in the respirometer when they emerged from the host puparia

without being fed. The respirometer leaves a record on ticker tape in which a hole is punched each time the apparatus accepts an offering of O_2 and another every 30 sec. to provide a time trace. The number of holes in the first trace divided by those in the time trace are a fraction of 10 cu. mm. per hour. In this way an absolute value for O_2 consumed over several days can be obtained. Females survived in the respirometer in some instances for as long as 5 days but it was difficult to get males to survive for 3 days. The results are presented in Table 2.

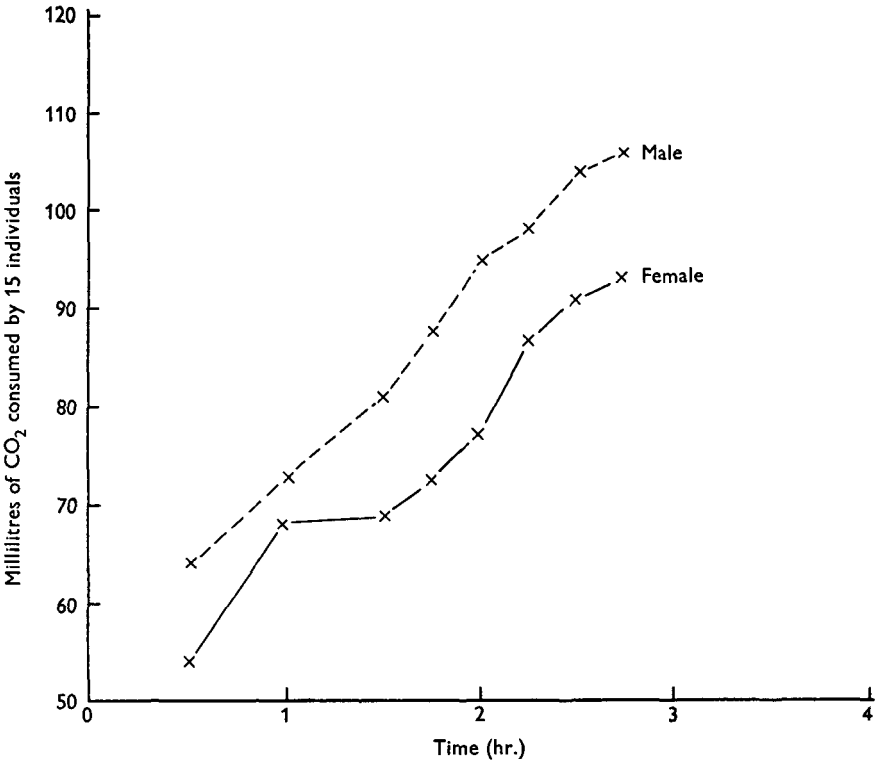


Fig. 6. Comparison of the output of CO_2 by the sexes of *N. vitripennis*.

Table 2. *Mean daily values of* O_2 *consumption (cu.mm./hr.) for the duration of the experiment*

	Female Individuals				
	1	2	3	4	5
Day 1	0·28	0·16	0·58	0·22	0·27
Day 2	0·24	0·22	0·25	0·16	0·26
Day 3	0·24	0·24	0·35	0·16	0·28

	Male Individuals			
	1	2	3	4
Day 1	0·53	0·19	0·16	0·22
Day 2	0·38	0·22	0·13	0·19
Day 3	0·31	0·14	0·13	0·14

B. Investigation of possible auxiliary food reserves in females of N. vitripennis

In this experiment some internal organs of both sexes were measured in starved and fed individuals to find if any part is resorbed which might supply extra nourishment to the female but not the male.

The sexes were separated when they emerged from *Lucilia sericata* and each sex was divided into two groups. One group of females was starved and the other was given honey smeared on the inside of the tube. After 2 days they were killed with ethyl acetate and dissected. An eyepiece micrometer was then used to measure the following structures: head width, total body length, maximum width and length of crop and hind gut, length of Malpighian tubules, diameter of dorsal muscles, diameter of fat cells, length of the ovaries and width and length of rectum.

Table 3. *Measurements of various organs in fed and starved* Nasonia vitripennis *of both sexes in mm.*

Each figure is mean for 10 specimens.

	Fed females	Starved females	Fed males	Starved males
Width of head	0·608	0·600	0·5536	0·5904
Total length	2·2208	2·04	1·9200	2·0112
Length of abdomen	1·0800	—	0·8720	0·9280
Crop length	0·4624	0·2976	0·4560	0·3246
Crop width	0·4910	0·3584	0·2576	0·2800
Stomach length	0·2528	0·2528	0·2096	0·1392
Stomach width	0·1696	0·1584	0·1088	0·0880
Hind gut length	0·4400	0·4816	0·4320	0·4928
Hind gut width	0·0672	0·0512	0·0529	0·5280
Malpighian tubes length	0·8608	0·7936	0·6720	0·6368
Diameter dorsal m's	0·1184	0·1376	0·0773	0·0720
Alary m's diameter	0·0624	0·0544	0·0400	0·0416
Rectum length	0·2496	0·2688	0·2640	0·2512
Rectum width	0·2528	0·1520	0·1968	0·2288
Ovary length	2·1744	1·0800	—	—
Testis length	—	—	0·1360	0·1648
Vesicula seminalis	—	—	0·1120	0·1168

These measurements (Table 3) showed that the width and length of the crop was smaller in starved than in fed specimens in both sexes but the hindgut was longer, probably because shrinkage of the crop was greater than that of the abdomen, thus stretching the hind gut. There was a slight reduction in the length of the Malpighian tubules, but some atrophy of these might be expected to accompany reduction in excretory products following starvation. Statistically, fat cells were not significantly less in starved specimens, but the ovary was significantly shorter ($t = 3{\cdot}72$, $P < 0{\cdot}01$).

The conclusion from these results is that the only major change in organs of starved individuals, when compared with fed individuals, which can have any bearing on the problem being studied here is the marked reduction in length of ovaries, from which some or all of the mature eggs have presumably been resorbed. It has been demonstrated that eggs are resorbed after 1–2 days of host deprivation, so this nutriment will be available (Edwards, 1954; King, 1959). Egg development continues even in the absence of host puparia (although under certain circumstances at a

slower rate); hence some nutriment which was already present in partly developed eggs at the commencement of host deprivation will subsequently become available. From the results presented in Figs. 1–4 it can be seen that large females outlive smaller females and although this suggests added supplies in the fat cells it is possible that more eggs are produced in large females than small females and this would help survival if the eggs undergoing resorption are an important source of reserve nutriment when the insect is starved.

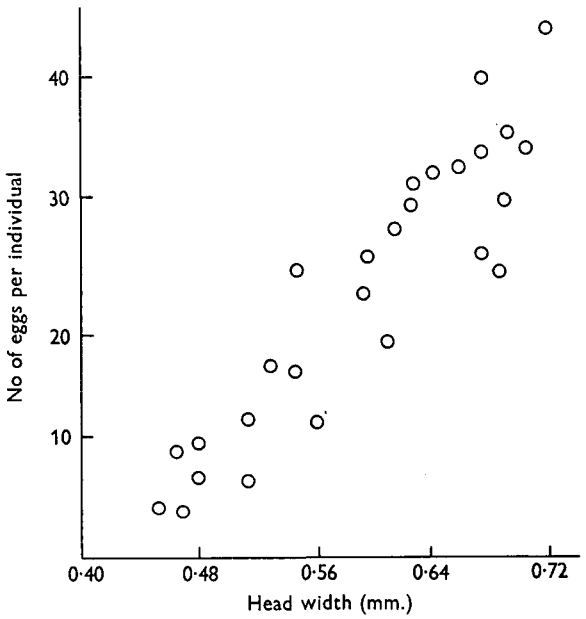


Fig. 7. The relation between the head width of *N. vitripennis* and number of mature eggs present in the ovaries.

Size of the female of N. vitripennis *compared with the number of mature eggs in her ovaries*

Females of *N. vitripennis* as they emerged from the puparia of *L. sericata* in which they were reared were provided with equal sized host puparia for one day in which they could oviposit and on which they could feed. At the end of this period the parasites were killed with ethyl acetate, dissected, and the number of eggs possessing a chorion in the ovaries was noted. There was found to be a correlation between the head width of the parasite and the number of eggs present in its ovaries ($r = 0{\cdot}91$, $P < 0{\cdot}01$) (Fig. 7).

DISCUSSION

The evidence indicates that, as in *H. juglandis* (Grosch, 1950), the females of *N. vitripennis* live longer than the males when both are starved, irrespective of size or of whether mating has occurred, the larger individuals of each sex outliving the smaller. This cannot be the result of more rapid desiccation of the smaller males than the larger females, or of the smaller individuals than the larger in each sex, because similar results were obtained when the experiment was conducted at a relative humidity of

100% where desiccation should not occur. The females of *Habrobracon* are more resistant to X-rays and to feeding with radioactive isotopes than the males (Grosch, 1956). It is possible that males are generally less viable than females in a haplo-diploid system. Because of the haplo-diploid sex-determining system (in *Nasonia*) the males cannot be heterozygous and it is possible that they are in general less fit than the diploid females. A difference in viability is explicable in physiological terms. The effect of different feeding methods in the two sexes was eliminated because in most experiments both were starved from the time of emergence from the host puparium in which they had developed, so that a possible residual effect in the form of stored food resulting from feeding before the start of the experiment was eliminated as far as practically possible. The investigation was then continued to try to explain the difference in longevity in physiological terms.

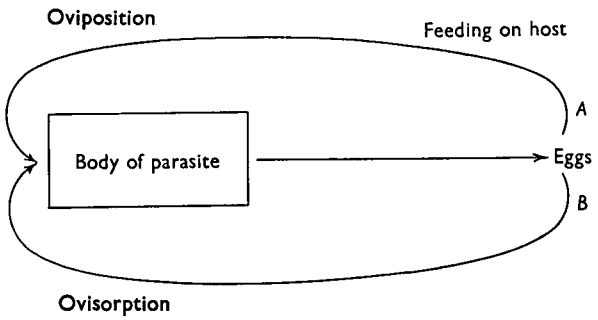
No significant difference was found between the rates of O_2 uptake by the two sexes using Macfadyen's continuously recording respirometer over a period of 24 hr. ($t = 0{\cdot}004, P > 0{\cdot}5$). The results obtained using Warburg manometers supported this. This eliminates the possibility that males are respiring at a more rapid rate than the females and so using up any food reserves more rapidly. Although Grosch (1950) suggested that this might in fact be the situation he did not support this portion of his work with experimental evidence.

The conclusion from these results is that the females must have an additional supply of food as compared with the males. After 3 days in Macfadyen's respirometer there was a significant difference in the respiratory rates of the sexes, the male being lower than the female, whose respiratory rate had not changed significantly over 3 days ($t = 4{\cdot}39, P < 0{\cdot}01$) (Table 8).

Because of considerable individual variation in respiratory rate statistical analysis cannot show significant changes, but examination of the daily means of hourly readings shows the trend in all individuals.

Various organs in starved and fed specimens of both sexes were measured and the only organs which showed a significant reduction in a starved specimen of either sex compared with a fed one were the ovaries. It has been demonstrated previously (Edwards, 1954; King, 1959) that egg production continues in the absence of hosts and under starvation conditions, though at a lower rate. The smaller size of the ovaries of starved specimens is due to the slowing of egg production and to egg resorption. During resorption nutrients from the yolk of eggs undergoing resorption pass into the body of the female and it is possible that these resorbed eggs provide the female with an extra food source. Edwards (1954) showed that within 1 or 2 days of host deprivation eggs undergoing resorption are present in the ovarioles. This has been confirmed and the exact time taken for an egg to be resorbed has been examined using colchicine (King, 1963). The males survive longer than 2 days when starved without having the possibility of resorbing eggs, so it is possible for starved females to survive long enough for the nutrient in the egg yolk to become available by the process of resorption. This nutriment may be at least one of the factors enabling a starved female to survive longer than a starved male, and larger females than smaller, since the larger individuals have more eggs present and thus available for resorption than do smaller individuals, there being a correlation between size of the female and the number of eggs present in the ovaries ($t = 0{\cdot}91, P < 0{\cdot}01$).

The female metabolic cycle can be represented as follows:



Under normal conditions with host puparia available continuously the metabolism will be along path *A* with *B* not functioning, but after two days' deprivation of host puparia, during which path *A* is closed, path *B* will open and a supply of nutriment will be made available to the insect.

SUMMARY

1. At relative humidities of 65 and 100% the mean length of life of virgin or mated females of *Nasonia vitripennis* is greater than that of the males under similar conditions.

2. The sexes showed no significant difference in their respiratory rates, as measured by Warburg manometer or Macfadyen's continuous recording respirometer.

3. An examination of the organs of fed and starved individuals of both sexes showed that the only major change in the organs of starved individuals which can have any bearing on the length of life is the marked reduction in length of the ovaries resulting from resorption of the mature eggs.

4. It is suggested that the resorbed eggs supply the female with the nutriment which enables it to outlive the male when both are starved.

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