

**INVESTIGATION OF *EMBLICA OFFICINALIS* DIET ON
LONGEVITY, BEHAVIOR AND FITNESS CHARACTERS
IN A *DROSOPHILID*: *PHORTICELLA STRIATA***

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ABSTRACT: *Drosophilid* is one of the model organisms to test Ayurvedic medicines. The objective of our study was to explore the potential of *Emblica officinalis* drug on longevity, sexual behavior and reproductive fitness of *Drosophila melanogaster* using adult feeding method. Increase in the lifespan, fecundity, fertility, ovarioles number and developmental time was observed in both parents and F₁ generation, but not in the F₂ generation in experimental culture (control+ *Emblica officinalis*). According to the DMRT and ANOVA there is a significant difference between two cultures. It was also noticed that *Emblica officinalis* influence some fitness characters in *Drosophila* along with sexual behavior.

KEY WORDS: Ayurveda, *Drosophilid*, *Emblica officinalis*.

Ayurveda-literally "Science of Life"-is based on the twin principles of wholeness and balance. As a holistic healing tradition, Ayurveda recommends treating the "whole" person-body, mind, senses, emotions and spirit-instead of following the "one-cause-one-cure" principle and focusing on the symptoms of the moment. The promise is that if the roots are nourished and watered, the plant will flourish. Diet, sleep, lifestyle, daily and seasonal routines and internal cleansing are just as, or more, important than herbs and potions in order to maintain good health. Ayurveda is equally about maintaining or preserving good health as it is about treating disorders - "*Swaasthasya Rakshanam*" is one of the goals of Ayurveda (Charaka Samhita Sutrasthana, 2000a).

The branch of *rasayana* or rejuvenation is one of the eight specialized branches of Ayurveda that primarily deals with the maintenance of health (Sushrutha Samhita Sutrasthana, 1972a). *Rasayana* is defined as any herb, food, or activity which confers youthfulness and cures diseases. If taken in a proper way the *rasayana* prevents early aging and keeps person young and active both physically and mentally (Charaka Samhita Chikitsasthana, 2000b). The literal meaning of *rasayana* is "augmentation of *rasa*", the vital fluid produced by the digestion of food. *Rasa* provides nutrition, enhances the immunity, and sustains life. *Rasayana* is the method of treatment through which the *rasa* is maintained in the body. The purpose of *rasayana* is to give strength, immunity, *ojus*, vitality, will power and determination, and to strengthen the sense faculties, so that you are not exposed to sickness and disease as long as you live.

According to Sushrutha Samhita Sutrasthana (1972b) Sexuality and reproduction is so vital in Ayurveda that an entire discipline, known as Vajikarana, is dedicated to enhancing fertility and the rejuvenation of sexual and reproductive energy. Vajikarana therapy improves the function of the reproductive organs and vitalizes reproductive tissues, increasing semen count and strengthening their motility and making eggs more viable for conception. This not only enhances the quality and longevity of individual life, but also the health and vitality of offspring. While some Vajikarana herbs work as

aphrodisiacs, they also engender reproductive strength in order to increase the health of our offspring, or what Vajikarana calls subahupraja – children who are physically and mentally vital and have the fundamental qualities needed for a conscious life Charaka Samhita Chikitsasthana (2000c). *Emblica officinalis* is such herb used as rasayana in treatment of diseases. However effect of this drug on normal life activities has not been significantly validated. Therefore the present study was carried out with an *objective* to explore the potential of *Emblica officinalis* drug, on fitness of *Drosophila bipectinata* using adult feeding method. We have *Drosophila melanogaster* as the test system which proved to be an excellent organism to test the effect of many drugs and other chemicals.

The fruit fly *Drosophila* is one of the most intensively studied in medicine and serves as a model system for investigations of many developmental and cellular processes common to higher eukaryotes, including man. This fly is being used for genetic studies since almost a century. Studies on *Drosophila* have enabled biologists to make significant contributions to the fields as diverse as basic genetics, population biology, evolutionary biology, behavioral biology and molecular biology. It has a short life cycle, easy and inexpensive in culturing and they could be kept in large numbers. Library of several *Drosophila* species with mutant and transgenic stock is available at different laboratories in the world. Presence of giant polytene chromosome and less number of the chromosomes are added advantages of the flies for genetic studies. Its genome has also been sequenced recently (Adams et al., 2000). Vertebrates have about four homologous for every gene found in *Drosophila*. The species shares large numbers of homologous genes with mammals, 13,601 with humans. These have been analyzed to identify sequences related to those causing human diseases (Reiter et al. 2001). The evolutionary conservation of gene function between humans and *Drosophila* make it an ideal model system for the study of the molecular mechanisms of human disease. Moreover identification of specific genes that regulate life span in *D. melanogaster* has been achieved by two processes: I. mutational analysis (Ford and Tower, 2006) in which manipulation of pathway function or gene has demonstrated life span extension. II. Quantitative trait locus analysis (QTL) (Tatar and Yin, 2001) in which genetic elements affecting natural variations in longevity have been mapped to specific positions along the chromosomes (Paaby & Schmidt, 2008, 2009). In *Drosophila* and many other insects body size is positively correlated with mating success, longevity, fecundity and other fitness characters and best phenotypic heritable characters (Santos et al., 1992). All these findings demonstrated the advantage of size in mating success and fitness. Various workers demonstrated the fitness studies in *Drosophila* such as fecundity, fertility (Long et al., 1980) and longevity (Cordts & Partridge, 1996, Partridge Tower, 2008).

Survey of the literature shows (Annalise & Paul, 2009) on decrease gene activity such as *chico*, *dlip genes*, *dS6K*, *dTOR*, *DTS-3*, *EcR*, *Indy*, *InR*, *mth*, *ovo*, *Puc* extends the lifespan in *D. melanogaster*. Simultaneously there are such as genes *Cat*, *CCT1*, *dFOXO*, *DPOSH*, *dTSc1*, *dTSc2*, *EF-1a*, *fwd*, *G6PD*, *magu*, *Mel-41*, *Pcmt*, *PTEN* which increase gene activity leads to lifespan extends. Moreover with these genes (*Catsup*, *Ddc*, *Dox-A2*, *Lim 3*, *stc*) allelic variation is also varies longevity of fruit flies. These facts has proved *Drosophila* one of the most excellent eukaryote for exploring the genetic determination of life span, genetics of degeneration and aging process, reproductive capacity. With these results there is an insight into what to seek in man. For instant the human *Xist* gene and the analogous *Drosophila Sxl* gene both control sex determination, and may both be involved in regulating longevity. This shows that any investigation *Emblica*

officinalis on the aging process on *Drosophila* will be fruitful research. In order to validate *Emblica officinalis* concepts more precisely, we proposed using *D. melanogaster* as a model. Till here there are very less literature is available for evaluation of some Ayurvedic medicine on *Drosophila* on life span *Emblica officinalis* fruit is one of the Ayurvedic drug used in *Rasayana* Chikitsa. But there is no literature on non *Drosophilids* such as *Phorticella striata*. Ayurveda lexicon states that the fruit of *Emblica officinalis* is the best medicine to increase longevity. The biological effect of this drug has not been tested in recent times, but only is being prescribed to human beings. Furthermore, assessing its effect in human beings has practical and ethical problems. Therefore to be revalidated the concept of *Rasayana* Chikitsa and in particular to be assesses the effect of *Emblica officinalis*. The present study has been carried out using *Phorticella striata* which belongs to *Drosophilids* group and non *Drosophila* as the test system. We authors therefore formulated a rasayana from *Emblica officinalis* organic herb and with traditional principles; this is carefully altered to reflect intrinsic differences between mammals and insects. Here, we tried to investigate the impact of the *Emblica officinalis* on fitness parameters such as number of fertility, fecundity, ovarioles and developmental time in *Phorticella striata* flies in next generation along with behavior and its fitness characters.

MATERIALS AND METHODS

Preparation of cultures

To study the impact of *Emblica officinalis* on some quantitative sexual behavior and fitness parameters *Phorticella striata* (Mysore strain), flies were caught from Chamundi hill from the nature. The pure culture of these flies was maintained under standard food medium (Guruprasad et al., 2010). The effect of *Emblica officinalis* was studied by adult feeding method. For this purpose the stocks of the flies were built up for five to six generation from isofemale line. The virgin females and bachelor males emerged from the normal media were isolated under ether anesthesia within 3 hours of eclosion. They were transferred to 8×2.5 cm glass culture vials containing equal quantities of normal food media used for *Drosophila* culture (wheat cream agar medium this medium was prepared by boiling 1000ml of distilled water along with 100g of jagery (sugar). When jagery dissolved in it, 100g of wheat powder (soji or rava) was added to the medium and then 10g of agar agar and 7.5ml of propionic acid (anti-fungal) were added gently). The medium was distributed to glass vials of 8×2.5 cm size. The mouth of the bottles/vials was kept closed with cotton. One day later one or two drops of yeast solution were added to the food media. This medium was used after 24 hours. At every step heat vials were used for preparing medium. This was to prevent outbreak of pests and diseases. Similarly sterilized cotton was used to plug the vials. This culture is used as control culture. For experimental culture 5 drops of *Emblica officinalis* was included to above wheat cream agar medium and virgin females and bachelor males were transferred into this media and aged for 4 days. There is no dietary restriction for the flies in both control and experimental culture.

Experimental Design

For longevity (life span) twenty five virgin females and bachelor males from both cultures were used to test the longevity. For this purpose each pairs were transferred to control culture and experimental culture. Daily these flies were

transferred to the fresh vials of both cultures until flies die from the day of eclosion. This is calculated in terms of number of days.

For analysis of sexual behavior patterns fifty virgin females and fifty bachelor males emerged from the normal media were isolated under ether anesthesia within 3 hours of eclosion and maintained them separately in both control and experimental culture for 4 days. Twenty five flies of both the sex were used to study some courtship activities (Spieth and Ringo, 1983, Hegde Krishnamurthy, 1979) such as mating latency, copulation duration. For observation of sexual behavior a virgin female and bachelor male were introduced into an Elens-Wattiaux mating chamber (5 cm x 5 cm circular glass chamber with a lid to facilitate easy observation). Because maximum mating occurs during morning hours, observation was made between 7 and 11 a.m. Mating latency (time between introduction of males and females into mating chamber and initiation of copulation of each pair), copulation duration (time between initiation and termination of copulation of each pair) were recorded. The terminologies are used as per the description of Hegde and Krishnamurthy, 1979). A minimum of 25 pairs involving each isofemale line were observed for both culture groups. Similarly mating latency and copulation duration was also observed in F₁ and F₂ generations (fed with normal food media) which were generated from the above 25 mated pairs which are considered as parents.

The reproductive fitness parameters such as fecundity, fertility, ovarioles number and developmental time were analyzed from mated parents and their two successive generations. For fecundity test mated males were transferred to vials containing normal food media and allowed to lay eggs for 24 hours. After 24 hours, the flies were individually transferred to fresh vial containing food media. The number of eggs laid (fecundity) during the following ten days was scored using stereomicroscope for both control and experimental groups. Twenty five replicates were maintained for each of the control and experiment studies. The fertility was measured by counting the number of the progeny produced by a single mated female. For testing fertility, each mated female was kept in an individual food vial for a period of one day and then transferred to a fresh food vial every day. Ten successive changes were made and the total number of flies that emerged from each vial was counted. Twenty five replicates were maintained for each of the experimental and control under study. Data were pooled and the mean number of flies per female was calculated. Counting of ovarioles number was done by selecting the twenty five virgin females separately and maintained in both control and experimental cultures and aged 4 days. These virgin females were dissected for left ovaries in saline and bundles of ovarioles were separated by fine needle and counted under a stereomicroscope. For analysis of developmental time, after emergence of the flies were counted every day from the first to last day of the eclosion. A minimum two pairs of virgin female and bachelor's male were selected from the all ten vials which are considered as F₁ generation. Similarly for F₂ generation flies were selected from the F₁ generation. These were mated separately maintained in the separate vial to calculate their fecundity, fertility, developmental time and ovarioles number too. All the culture was maintained under the laboratory condition at a temperature 24±2°C. One way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was applied for all parameters using SPSS 10.5 software.

RESULTS

The data on the longevity (lifespan) is represented in Table 1 reflects longevity of *Drosophilids Phorticella straita* where the parent flies fed with experimental culture shows high lifespan with 72 minimum days and 83 maximum days than F₁ generation, F₂ generation and control one too.

The data on sexual behavior and fitness parameters of *Phorticella straita* of two different cultures has been reported in table 2. Sexual activities such as mating latency was highest in control culture than experimental culture (10.32±0.72) and it is significant by one way ANOVA and DMRT (F value = 20.04; P<0.001) between the two cultures this is also seen in their successive generations. Mean while copulation duration is highest in experimental culture (28.64±1.15) compare to control culture this is also carried in the nest generation with highly significant according one way ANOVA and DMRT (F value= 30.45; P<0.001) which is opposite to copulation duration.

The scrutiny of table 2 also shows fitness parameters such as fecundity, fertility, developmental time and ovarioles number was high in experimental culture compare to control and significant too between two cultures (fecundity=131.1±1.36, fertility=122.9±1.32, developmental time=15.63±0.15, ovarioles number=17.00 ± 0.73, F value=24.85; 16.73; 6.57; 15.75; these mean values are more compare to their F₁ and F₂ generation. Table 2.represents mean values of fitness parameters, where F₁ progeny shows high compare to F₂ generation (fecundity=128.1±1.21, fertility=120.3±1.47; developmental time 14.04±0.13; ovarioles number=15.00±0.71). All these parameters are highly significant when compare between the cultures (table 2). The above fitness parameters and sexual behavior are depicted in figure 1 and figure 2, where flies reared in control media shows no such variation among the parameters, but there is variation in the mean values in the experimental culture between the parents, F₁ generation and F₂ generation.

DISCUSSION

For the above results the longevity is more in case of parents compare to their progenies in experimental cultured fed flies than control one this confirms there is influence of *Embllica officinalis* which is mixed in experimental culture. These results were similar to some workers Charaka SamhitaSutrasthana.2000a. Sexually reproducing animals are endowed with special features, first to produce fertile offspring and second to adapt to a particular environment. The reproduction is preceded by a series of courtship acts where in males and females show unique rituals to attract each other, mate and produce the offspring. The courtship and mating although are genetic, are also influenced by various factors. This may imply that these courtship activities are directed by the same set of genes and that these traits are related genetically. This agrees with the observations which show the genetic determination of certain components of sexual behavior in *Drosophila* (Spieth & Ringo, 1983; Spiess, 1970; Parsons, 1973). Though genetically determined there is every possibility for a change in sexual behavior because these activities are also influenced by changes in physical environment according to Crossley, 1974; Gutzke and Crews, 1998; Laudien, et al., 1980; Sisodia and Singh 2002; West and Packer, 2002). In the present study an effort is made to study the effect of *Embllica officinalis* on quantitative sexual behavior and fitness parameters on *Phorticella striata*. From our studies, the mating latency was shorter in the flies reared in experimental culture than either

at high in control (Table 1). The differences in mating latency in two cultures were also statistically significant (by ANOVA and DMRT). Mating latency is measured as the time taken for the male oriented towards female until initiation of copulation duration by Markow, 1985. It is the period during which the pairs acclimatize in the mating chamber and then start the courtship activities. It actually indicates the vigor of male (Eastwood & Burnet, 1977). A male with high vigor reacts quickly in the presence of female while a male with less vigor reacts slowly (Markow, 1988). Obviously shorter mating latency indicates higher vigor of male. The shorter mating latency was noticed in experimental culture. This suggests that the males from experimental culture have higher vigor and therefore are quickly attracted by the females. The mating latency was also shorter in the flies from experimental culture compared control shown in figures 1 and 2. The Mating latency not only indicates vigor of males and but also receptivity of females. It is the time required for males and females to initiate copulation. Higher the vigor of males and receptivity of females, shorter is the mating latency. During this period, courtship acts are performed mostly by males, to increase the receptivity of females and to make her sexually excited (Spieth & Ringo, 1983). A male with high vigor has to perform the same courtship act more number of times to a non-receptive female than to a receptive female. If she is receptive, only a few courtship acts are performed leading to quick pairing. The short mating latency of experimental culture in the present studies therefore indicates that the males maintain high vigor and females maintain high receptivity at this culture while at those in control, they cannot maintain the vigor and receptivity. Mating latency of control culture of parents and their generations more or less equal (Table 2).

Courtship activity of the male or female culminates in copulation demonstrated by Spiess (1970). During copulation sperms from the male is transferred to the female reproductive tract and therefore the duration of copulation has a lot of significance in an animal's life. In the present studies, the copulation duration was longest at experimental culture than at control one. According to Guruprasad, et al. (2008) longer duration of copulation permits the transfer of more number of sperms by male to the female. Therefore extension of copulation duration enhances the fitness of the male. It can also enhance the fitness of the females because the sperms received by a female can fertilize more number of eggs. Therefore it is unlikely that the longer copulation duration could enhance the fitness. As the females have high receptivity and males have high vigor.

In *Drosophila*, The fecundity remains one of the less known quantitative traits along with fitness parameters such as fertility, developmental time and ovarioles number. Estimation of fecundity and fertility is important in routine testing of various chemicals. This gives an insight into the extent of effect on ovarioles and other physiological factors, which is expressed in the terms of egg and offspring production. Table 2 reveals the mean fecundity eggs / female in the adult feeding methods was more than control. According to ANOVA and DMRT have shown that increased fecundity in experimental were significant ($P < 0.001$). This indicates that the mode of administration is also important factor, which one should consider while assessing the effect of any chemical on any biological system. In contrast to this in control culture, the decrease in fecundity may be accounted for the fact that the flies are under the influence of *Emblica officinalis*, hence they might not have been able to lay eggs rather than producing less egg. This finding agrees with the observation of Gruwes et al. (1971) where they noticed oviposition rhythm in *Drosophila melanogaster* and Vogel (1972) has demonstrated that certain aziridine analogous have discernible effect on fecundity

in *Drosophila*. Table 1 also incorporates mean fertility per female in adult experimental diet with *Emblica officinalis* was seriously increase compare to control, and they were more fertile than control, ($P < 0.001$ by ANOVA and DMRT). Several workers have made studies on the effect of different chemicals on fertility in *Drosophila melanogaster* (Vasudeva and Krishnamurthy 1983). The present study of the author agrees with them that influence of the chemicals will alter the fertility in *Drosophila*.

Rate of development is another parameter, which is used to analyze the some chemicals clinically. In the present investigations the genetic constitution, amount of the food, temperature, and space were kept constant. Obviously the differences in the developmental must have been determined by the chemical used or not by the other factors. This type of effect on the developmental time by different chemicals in *Drosophila melanogaster* has been shown by Luning (1966).

The estimation of fitness is the first step in understanding the adaptive evolution of a population. Ovarioles number is an anatomical trait determined during pupation for which a polygenic basis is known in various species of the *D. melanogaster* complex. Interestingly, our results are contrary to this where experimental culture fed flies are having more ovarioles than control this shows there is influence of the *Emblica officinalis* rasayana mixed in experimental culture.

Figure 1 suggests that all the fitness parameters and sexual behavior of *Phorticella striata* reared in control media is similar in all parents and their generation too. According to Figure 2 fitness parameters and sexual behavior of *Phorticella striata* in experimental media have variation among F_1 generation and F_2 generation compare with their parents. The Flies which are fed in the experimental culture have long life span than control one (Table 1). Our results confirm the effect of *Emblica officinalis* on parents than their progenies and the impact of *Emblica officinalis* is carried to F_1 generation rather in F_2 generation in experimental culture. The author in this study observed variation in the developmental time in both cultures (Table 2) longest mean of developmental time was noticed in the experimental cultures.

CONCLUSION

The conclusion of our experiment suggests that rasayana of *Emblica officinalis* enhance the sexual activities. This obviously increases in longevity, fertility, fecundity, ovarioles number along with developmental time. The Influence of the above rasayana is found in the parents and F_1 generation and not in the F_2 generations. The impact of the rasayana is not carried in further generation after F_1 generation Lastly, author concludes there is also linear interrelationship between sexual activities and fitness parameters in experimental culture. "Adding Life to years is better than adding years to life". So, along with longevity other reproductive fitness characters of flies were undertaken for this study so as to explore the hidden principles of rasayana therapy which improves the quality of the life.

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Table 1. The longevity of *Phthoricea straita* cultured in control and experimental media.

	Culture	Parents (Min/Max)	F1 Generation (Min/Max)	F2 Generation (Min/Max)
Longevity	Control	40/56	43/53	42/54
	Experimental	72/83	69/74	56/65

Min-minimum days; Max-maximum days

Table 2. Sexual activities and fitness parameters of *Phorticella straita* (values are Means \pm SE).

Parameters	Culture	Parents	F1 Generation	F2 Generation
Copulation duration	Control	19.76 \pm 0.62	18.40 \pm 0.42	20.41 \pm 0.76
	Experimental	28.64 \pm 1.15	26.42 \pm 1.17	23.32 \pm 2.16
	F= value	30.45**	27.15**	25.16**
Mating latency	Control	10.32 \pm 0.72	10.14 \pm 0.82	9.14 \pm 0.42
	Experimental	7.20 \pm 0.85	7.45 \pm 1.06	9.02 \pm 1.46
	F= value	20.04**	18.16**	15.23**
Fecundity	Control	118.3 \pm 1.43 ^a	116.3 \pm 1.22 ^a	115.3 \pm 1.43 ^a
	Experimental	131.1 \pm 1.36 ^b	128.1 \pm 1.21 ^b	121.1 \pm 1.19 ^b
	F= value	24.85**	23.65**	20.51**
Fertility	Control	106.3 \pm 2.48 ^a	104.2 \pm 3.34 ^a	102.8 \pm 1.03 ^a
	Experimental	122.9 \pm 1.32 ^b	120.3 \pm 1.47 ^b	113.4 \pm 1.35 ^a
	F= value	16.73**	15.43**	13.04**
Developmental time	Control	11.65 \pm 0.09 ^b	11.43 \pm 0.11 ^a	10.02 \pm 0.09 ^a
	Experimental	15.63 \pm 0.15 ^a	14.04 \pm 0.13 ^a	11.03 \pm 0.13 ^b
	F= value	6.57*	5.06*	6.46*
Ovarioles number	Control	14.00 \pm 0.13 ^a	13.00 \pm 0.12 ^a	13.00 \pm 0.10 ^a
	Experimental	20.00 \pm 0.73 ^b	16.00 \pm 0.71 ^a	13.00 \pm 0.62 ^c
	F= value	15.75**	13.04**	14.04**

Different alphabet as superscript in each column is significant according DMRT.

*P<0.01; **P<0.001;

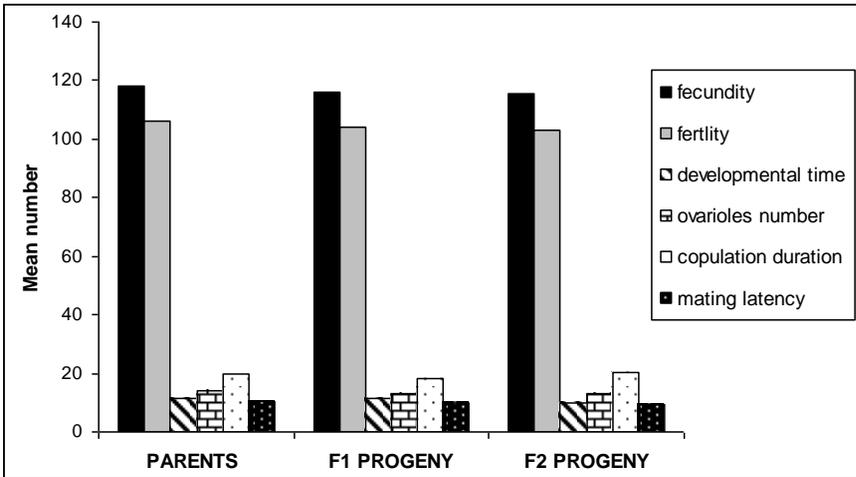


Figure 1. Fitness parameters and sexual behavior of *Phorticella straita* cultured in control media.

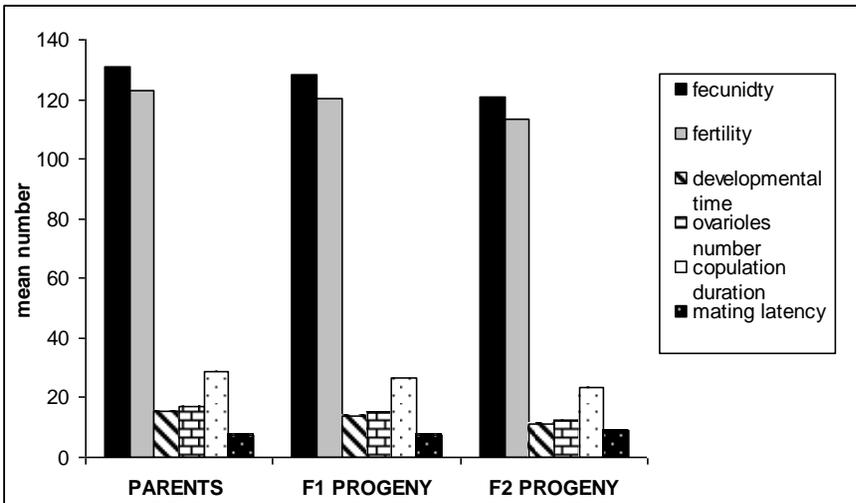


Figure 2. Fitness parameters and sexual behavior of *Phorticella straita* cultured in experimental media.