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A NUTRITIONAL STUDY OF INSECTS, WITH SPECIAL REFERENCE TO MICROÖRGANISMS AND THEIR SUBSTRATA[1]

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[1] Contribution from the Entomological Laboratory of the Bussey Institute, Harvard University.

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INTRODUCTION

Throughout the whole organic world the essential food element most difficult to acquire is nitrogen, as all nitrogen must ultimately come from the atmosphere and the power of combining with this gas is limited to a few microörganisms. Upon the nitrifying bacteria, then, all higher plants and animals are dependent for their nitrogen which is handed from one organism to another, linking all together into one great interdependency which has

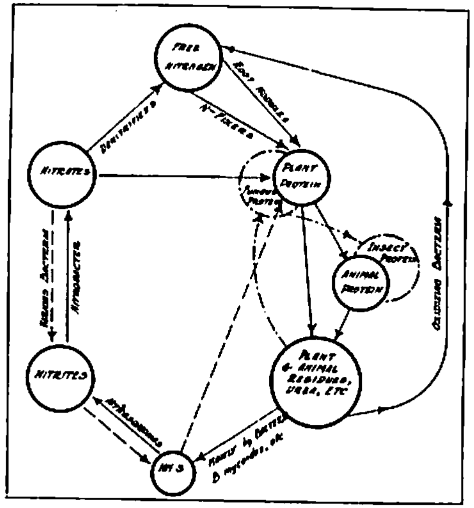

Fig. 1 The nitrogen cycle (from Bayliss). The accessory lines and circles in — - — - — are my additions based on evidence in this paper.

been called the nitrogen cycle. I insert a diagram from Bayliss which clearly illustrates this cycle. The accessory circles and the lines that connect them are additions based on my experiments.

The search of the insect for nitrogen is very complicated and has been, at times, obscure. Indeed, little definite information is at hand concerning the food requirements in general of these organisms, as the material consumed is often in large part merely the substratum for a small amount of assimilable food. This has led to many misunderstandings as to the synthetic power of

insects. Since they are largely phytophagous, insects are amply supplied with carbohydrates, but have difficulty in obtaining sufficient protein. The abundance of the former permits great activity, while the dearth of the latter limits the growth of the insect. This has led to a lengthening of the life-cycle in those species which must ingest large quantities of substrate in order to get enough nourishment to complete their growth. However, many insects that feed in decaying of fermenting vegetable matter of low protein content have an unusually short period of growth. The experiments and considerations which follow throw light on the protein supply of such insects and account for their rapid growth.

These investigations were made at the Bussey Institution for Research in Applied Biology, Harvard University, under Prof. W. M. Wheeler. Valuable advice and assistance were received from Profs. C. T. Brues, W. J. V. Osterhout, I. W. Bailey, and Dr. R. W. Glaser. I am especially indebted to Doctor Wheeler for helpful suggestions and encouragement.

EXPERIMENTS

1. *Food of an insect* (*Drosophila*) *living in fermenting fruit*

A. Method and initial observations. *a.* Solid media for Drosophila. While rearing Drosophila it was found necessary to determine the exact date of oviposition. As this is impossible in the ordinary culture tube of fermenting banana, a solid transparent medium was devised by myself and Dr. R. W. Glaser (1917 a).

This medium is made as follows: Mash six ripe bananas in 500 cc. of water, allow to infuse on ice overnight, strain through cheese-cloth, and add 1½ grams powdered agar-agar to each 100 cc. of the filtrate. Heat in double boiler till agar is dissolved, filter hot through absorbent cotton into test-tubes. Plug tubes, sterilize in autoclave, and allow to cool in inclined position so as to form solid slants of the medium.

This medium is quite transparent, affords 15 to 20 sq. cm. area for oviposition and 6 to 10 cc. of substratum for the larvae. The found that certain crystalline solids left on the filter after filtering autolyzed yeast could be substituted for lecithin. These crystalline substances are probably also constituents of yeast nucleoprotein.

Loeb and Northrop ('17) have recently used glucose beef agar for maintenance of larvae and adults so that the temperature coefficient of the duration of life could be determined, and Northrop ('17 a) has shown that the total duration of the life of Drosophila can be increased by retarding the growth of the larvae, as the pupal and imaginal periods do not seem to change with the increased larval life. These results are entirely comparable to those given on page 30. Northrop ('17 b) describes experiments which have led him to the conclusion that yeast supplies a special substance necessary for the growth of Drosophila larvae. This author finds that banana, casein, and sugar supplement yeast as a food for larvae and permit the development of a larger number of adults than could take place on yeast alone. The optimum mixture contained 33 per cent yeast, and as the amount of yeast decreased the number of adults reared became less and growth of larvae slower until at a proportion of yeast of 1:128 the growth of larvae became abnormal. Kidney, liver, and pancreas of dog were adequate foods for larvae, but spleen, heart muscle, muscle, blood, adrenal, and thyroid were not a complete diet for the insect. The author concludes that the special substance required for growth cannot be obtained from protein or carbohydrates. From my experiments I have evidence (p. 14) that banana and sugar have food value for Drosophila larvae, and to this extent my results are in accord with Northrop's, however, since the insects can develop normally on yeast nucleoprotein, sugars, and salts it seems probable that the special substances required for the growth of Drosophila are included in nucleoprotein.

In summing up the results of my experiment I conclude: 1. Drosophila normally feeds on fermenting fruit, obtaining a large part of its nourishment from the microörganisms, especially yeasts, which are in a loose symbiosis with the insect.

2. Dead or living yeast is a complete food for Drosophila.

3. The larvae are dependent on the nucleoprotein of yeast for special substances necessary in their growth.

4. The function of yeast in the ecology of the insect is to concentrate at the surface of the medium and to synthesize into nucleoprotein, the urates, ammonia, or amino-acids of the substratum.

2. Experiments with a sarcophagous insect

A pair of adult Acalyptrate muscid flies of the species Desmometopa m-nigrum Zett. (determination by Mr. C. W. Johnson) were received through the courtesy of Dr. W. M. Mann. They had emerged from some poorly dried snail shells, collected in the Fiji Islands, on the decaying flesh of which the larvae had fed. The adults were placed on banana and yeast agar, where the female deposited about forty eggs, most of which died owing to a thick mat of a black mucor that grew over the surface of the medium. The six larvae that emerged fed readily on the rich yeast food, and in about twenty-two days reached a size of 12 to 15 mm. in length. The black fungous mat was not destroyed and did not seem to injure the larvae. The six pupae formed were normal, and six adults emerged after three to five days and oviposited on the medium.

The usual manner of interpreting the normal feeding habits of this species would be to state that the larvae fed on decaying animal tissue. This, however, is open to doubt in view of the above experiments, and we must now consider the probability that all decaying or fermenting substrata are merely the media on which the fungus and bacterial food of the insect is growing.

3. Experiments with a coprophagous insect

An investigation of the food of the housefly (Musca domestica) also gives support to this theory. The insects were obtained in winter by placing bran mash in the greenhouse. The mash was prepared by boiling "Educator" bran with an equal volume of water with constant stirring for twenty minutes. It was placed in a large porcelain dish in the hothouse, where it soon became

EXTENT OF MYCETOPHAGY AMONG INSECTS

As a corollary to the foregoing conclusions we may assume that the foods of many insect larvae feeding on dead, decaying, and fermenting vegetable and animal matter are the micoörganisms which live upon the substratum in which the insects are embedded.

The extent of this habit among insects is very great, including a large number of Coleoptera and an especially large number of Diptera. This habit is usually apparent from the habitats selected by the insect, thus Metcalf ('16) lists the following habitats for the scavenger short- and long-tailed filth larvae of the flower-fly (Syrphidae), viz.: In decaying parts of trees and herbaceous plants, diseased or flowing sap, heaps of turf or soft mud containing vegetable matter, and in stagnant or putrid water, sewage, manure, or human feces. The larvae also occur as accidental body parasites, causing intestinal, nasal, auricular, and vaginal myasis. Some species serve as scavengers in the nests of termites, ants, wasps, and bees. It is apparent that microörganisms abound in all these environments, with the possible exception of the animal body In the latter case, however, it is well known that a foul odor, indicating some bacterial action, always precedes infestation. In more normal habitats the microörganisms so completely outweigh the other nutritive materials that it is quite likely they (the bacteria) serve as food.[21]

Townsend ('93) lists the following habitats for some of the scavenger Acalyptrate muscid larvae: dung, decaying wood, under bark, plants, leaves, roots, tubers, and fungi; in salt or alkaline water and mud; urine, vinegar, sap of wounded trees; cheese and animal fats. Again in this case all habitats selected by the fly normally abound in microörganisms, and it is quite safe to assume that they (the fungi) serve as food for the insect larvae.

The great extent of the use of microörganisms as food among insects is shown in a table of the feeding habits of larval and adult Diptera. In this table I have assumed that the food of insects, that always inhabit substrata of a fermenting or decaying nature,

[21] Osborne and Mendel ('14) showed that the bacterial content of feces was 20 to 40 per cent.

Group	Larval food				Adult food				
	Saprophytic fungi	Algae	Higher plants	Animals	Saprophytic fungi	Fermentation products	Higher plant juices	Nectar	Fluids of animals
Tipulidae	x	x	x		?	?		x	
Ctenophorinae	x						x	x	
Tipulinae	x							x	
Limnobiidae								x	
Cylindrotominae		x	x						
Limnobiinae	x	x			x			x	
Limnobia	x							x	
Pediciinae		x		x				x	
Limnophilinae	x	x							
Limnophila	x	x							
Eriopterinae									
Helobia	?							x	
Gnophomyia	?							x	
Hexatominae	x	x							
Trichocerinae									
Trichocera	x								
Ptychopteridae	x	x						x	
Rhyphidae	x				?	?		x	
Boletophilidae	x								
Mycetophilidae	x				?		x	x	
Leia	x								
Exechia	x							x	
Sciaridae	x							x	
Platyuridae	x							x	
Psychodidae	x	x			x	x		x	x
Blepharoceridae		x						x	x
Culididae	x			x			x	x	x
Dixidae		x						x	
Ceratopogonidae	x								x
Chironomidae	x			x				x	
Orphnephilidae	x			x					
Bibionidae	x							x	
Scatopsidae	x				x	x		x	
Simuliidae		x							x
Stratiomyiidae	x	x		x				x	
Stratiomia	x	x		x				x	
Odontomyia	x	x		x				x	
Oxycera	x	x		x					
Geosargus	x								
Microchrysa	x								
Eupachygaster			x	x					
Xylophagidae			x	x			x	x	
Coenomyiidae	x			x			x	x	
Tabanidae	x			x					x
Coniops	x			?					x

Group	Larval food				Adult food				
	Saprophytic fungi	Algae	Higher plants	Animals	Saprophytic fungi	Fermentation products	Higher plant juices	Nectar	Fluids of animals
Tabanus	x			x					x
Leptidae	x			x				x	
Atherix								x	
Chrysopila	x							x	
Cyrtidae				x					
Asiloidea				x				x	x
Mydaidae				x				x	x
Asilidae				x				x	x
Dasyllis				x				x	x
Bombyliidae				x				x	
Therevidae				x				x	
Scenopinidae	?			x				x	
Empidiae	x			x				x	x
Drapetis	x								x
Dolichopodidae				x				x	x
Phoridae	x			x					
Platypezidae	x								
Bipunculidae				x					
Syrphidae	x			x				x	
Conopidae				x				x	
Psilidae			x						
Sepsidae	x				?				
Trypetidae	x		x						
Sapromyzidae	x								
Agromyzidae			x						
Geomyzidae			x						
Drosophilidae	x				x	x			
Ephydridae		x	x						
Oscinidae			x						
Phycodromidae	x								
Borboridae	x	x			x	x			
Heteromeusidae	x								
Helomyzodae	x				x				
Cordyluridae	x		x	x					
Anthomyidae	x		x	x					
Muscidae	x			x	x	x		x	x
Oestridae				x					x
Sarcophagidae	x			x	x	x			
Dexiidae				x					
Tachinidae				x					
Hippoboscidae				x					x
Streblidae				x					x
Nycteribiidae				x					x
Sciomyzidae	x	x							

is the microörganisms themselves, and to a less extent the substratum. This assumption can safely be made on the basis of the preceding experiments and the general lack of nutritive value (for insects) of many of the substrata concerned. This interpretation of the food of scavengers, etc., has never been given before to the author's knowledge except in the case of Drosophila, as mentioned above (Guyénot, Loeb, Schulze, Henneberg). The data in the table are mainly derived from Malloch ('17) and Williston ('08). From a glance at the table it is apparent that a large majority of the Diptera live upon microörganisms. Therefore it might be well to include under the term Mycetophages all insects which have hitherto been termed scavengers, coprophages, etc.

Parrott, Fulton, and Gloyer ('14, '15, '16) found that tree crickets eat fungous "mycelia and spores which are unaffected by the intestinal juices."[25] This assumption is based on the fact that the spores of fungi are not digested by Oecanthus, as germination takes place from pellets of material passed through the digestive tract, and they believe

> it is possible that the spores may act as roughage and were eaten for that purpose; but it appears more plausible that the spores still retain on their surface some of the protoplasm of the ascus or pycnidium which makes them palatable ('16, p. 12).

The assumption that fungi are not digested, based as it is on the evidence that many living spores pass through the digestive tract, is not entirely justified in my opinion, since the same can be said of yeast cells in the Drosophila larvae, although in this case the plant undoubtedly serves as food.

The leaf-cutting ant is a good example of a mycetophagous insect. The fungus-growing habits of these Attiine ants should really be depicted at the same time as the habits of termites, as

[25] The wound made in tree twigs in which the eggs are placed is plugged with excrement and often becomes the seat of the development of cankers. Gloyer and Fulton ('16) give a concise account of the literature on the dissemination of plant diseases by insects. This is in large part due to the habit of insects of feeding on all available fungus and bacterial growths and in doing so getting the body covered with spores which readily remain attached to the hairs and spines on the surface of the body.

they are equally specialized. However, the origin of the habit appears to be different, as will be seen later, therefore their fungus gardening is best described at this point. Our knowledge of these forms is specially due to the work of Bates ('63), Belt ('74), Tanner ('92), and others. These ants excavate subterranean nests composed of a series of chambers connected by a vertical shaft usually ending in a crater. The fungus garden is built of the comminuted fragments of leaves, cut from trees near the nest, which have not passed through the digestive tract. The little pellets thus formed are built up into the sponge-like mass suspended from the ceiling or placed on the floors of the chambers. Caterpillar droppings are quite commonly built into the comb, which serves as a substratum for special kinds of fungi. A summary of all the literature on these ants, the termites and the ambrosia beetles, as well as some important contributions to the ethology of American species, is given by Wheeler ('07), from which I quote the following conclusions of Möller:

> All the fungus-gardens of the Atta species I have investigated, are pervaded with the same kind of mycelium, which produces the 'kohlrabi clusters' as long as the ants are cultivating the gardens. Under the influence of the ants neither free aerial hyphae nor any form of fruit are ever developed. The mycelium proliferates through the garden to the complete exclusion of any alien fungus, and the fungus garden of a nest represents in its entirety a pure culture of a single fungus. The fungus has two different forms of conidia which arise in the garden when it is removed from the influence of the ants. The hyphae have a very pronounced tendency to produce swellings or diverticula, which show several more or less peculiar and clearly differentiated variations. One of these, which has presumably reached its present form through the influence of cultivation and selection on the part of the ants, is represented by the 'kohlrabi heads.' Under artificial conditions the 'kohlrabi clusters' and 'heads' disappear and the fungus becomes a mass of bead-like conidia.

Sampaio ('94), von Ihering ('98), Goeldi ('05), and Huber ('05) have shown how the new fungus colony is started after the marriage flight. The queen deälates itself, digs a small subterranean burrow which it closes and then starts the new colony by spitting out a pellet of fungous hyphae which had been carried in the buccal pocket and depositing eggs upon it. The fungus

colony is grown upon the liquid excrement of the female, which touches small pieces of the mycelium to the tip of its gaster and then replaces them in the fungus garden. Some of the eggs are sacrificed as manure for the fungi.

Parrott and Gloyer ('16) are of the opinion that the fungus-growing habit of the Attii may have come about through the collection of caterpillar feces in which many spores accidentally eaten by the larvae are still alive. They do not give any explanation of the habit of collecting the feces, however. Wheeler ('07) points out that the gardens are usually composed of a substratum consisting largely of fecal material in the case of the ambrosia beetles and termites, and that the habit is pronounced in the lower genera of leaf-cutting ants and visible in all cases closely studied. It therefore seems probable that the food of the Attii "may have been originally grown on fecal substances" Von Ihering ('94) believes that the habit may have phylogenetically originated with ants using moldy seeds stored as food. I should suggest the possibility that the ants may originally have fed upon fungous mycelia developing on caterpillar feces from spores unkilled in their passage through the digestive tract. Such droppings might finally be carried into the nest where the fecal substratum and the moisture of the nest would soon allow the growth of a valuable crop of fungous food. Thus the fungus-growing ants developed their habits as a direct response to a valuable food supply. On the other hand, the termites developed their habits as a means of making use of an unmanageable food supply.

The examples cited above indicate that the use of microörganisms as food[26] is widespread among insects and is a direct response to the high food value of the fungous cells. The feeding habits may be grouped into three classes as follows:

1. Ingestion of microöganisms with substratum, i.e., Drosophila, Musca, Sciara, worker termites.
2. Feeding directly on microörganisms, i.e., mites, tree crickets, many adult Diptera, etc.

[26] See page 68 for case of mosquito larvae feeding on fungi.

3. Preparation of medium for microörganisms, i.e., leaf-cutting ants, termites, ambrosia beetles.

MICROÖRGANISMS AS LIQUEFIERS OF THE SUBSTRATUM

The relation of the insect, microörganism, and substratum is not always as clearly defined as in the preceding cases. Fabre ('94) studied the food of Lucilia, the green-bottle fly, and came to the conclusion that the larvae secrete a digestive fluid which allows the liquefied albuminous material to be sucked up by the insect. He placed in one tube of hard-boiled eggs a few fly eggs and left the other tube of albumin equally exposed to the air, as a check. The albumin on which the larvae emerged was soon a liquescent mass, whereas the check dried up. Guyénot ('07) reinvestigated the problem with Phormia regina Meigen and also studied the anatomy of the larvae. The mouthparts as he describes them are very similar to those of Drosophila larvae, as mentioned above. The pharynx is immediately connected with the crop of sucking stomach, a much distended flask-like structure which usually lies to one side of the oesophagus. Owing to the nature of the pseudomaxillary apparatus, the larvae are unable to eat any solid food. Fabre had supposed they secreted on the food some pepsin which liquefied the albumin. To test this theory, Guyénot ground up the larvae and made various extracts. The extracts had no effect on starch, fat, or albumin; the same was true of extracts of the salivary or gastric glands. The normal liquefaction was then studied, and it was found that the albumin was broken down to the peptone stage by a bacterium Micrococcus flavus liquefaciens (Flugge) which was always present with the larvae. The bacteria alone, without the acid of the larvae had the same effect on the albumin, but at a much slower rate. However, if they were mixed with the albumin with a sterile platinum wire the speed was as great as with the larvae present. As these bacteria were found in large quantities in the sucking stomach of the larvae, Guyénot reached the following conclusions:

I. La liquéfaction des substances albuminoides résulte d'une véritable digestion opérée par certains microbes de la putréfaction.
II. Les larves de mouches, absorbant exclusivement des aliments liquides, directment assimilables, ont un travail digestif réduit au minimum et ne produisent pas de ferments solubles en quantité appréciable.
III. Les larves accelérent la putréfaction des cadavres en favorisant la pullulation des microbes.
IV. Les larves se nourrissent aux dépens des produits du chimisme microbien; les microbes ne peuvent se développer rapidement que s'ils sont repartis en tous points par les larves. Il existe entre ces deux agents de la putréfaction une véritable symbiose (p. 369).

Guyénot does not consider that the food of the larvae may be the microörganisms themselves, and this question is still open, however, unlike the following example, the microörganisms associated with Lucilia have the function of liquefying the food material.

Bogdanow ('06) studied the similar case of Calliphora vomitoria, the flesh fly. The eggs were sterilized by washing for two one-and-one-half-minute periods in 5 : 1000 aqueous $HgCl_2$ solution and then rinsed in running sterile water and were then placed on sterile media of casein, egg albumin, etc. None of the flies obtained was sterile, but was usually associated with a micrococci which Bogdanow believed was passed through the egg. The larvae grew rapidly on casein, egg albumin, albuminoides, etc., in the presence of micrococci and a gelatin-dissolving bacterium, but the flies that emerged were few in number and very small in size, being 'starvation forms.' The larvae were later given the selection of fresh or putrid meat, showed a preference for the former, the putrid meat usually killing the larvae. Meats putrifying in the presence and absence of larvae could be distinguished by a difference in odor as the micrococcus with which the insect infects the meat liberates ammonia from proteins. The larvae grew normally on meat in the presence of a gelatin-liquefying bacterium and the micrococcus. Two factors are therefore necessary for the successful metamorphosis of the larvae the micrococcus from the egg and a gelatin liquefier from the air.

In 1908 (b) Bogdanow published a second paper on the same subject in which he showed that about 35 per cent of the eggs of the flesh fly are infested with a pure culture of micrococcus. The other 65 per cent are sterile and can be reared on nutrient gelatin in the presence of a bacterium capable of its liquefaction. These larvae seldom result in normal-sized adults and it was not possible to raise sterile larvae on a synthetic medium of meat ash, peptone, and meat extract, acid or alkaline. Therefore Bogdanow concluded:

1. Im einfach sterilisierten Fleische wächst die Calliphoralarve gewöhnlich sehr schlecht.
2. In sterilisierten Resten der Larvennahrung wächst sie nicht besser.
3. Für die gute Larvenentwicklung sind meistens gelatineverflüssigende Bakterien oder Trypsin nötig (p. 193).

As nutrient gelatin is a highly inadequate diet for Drosophila, it is probable that the Calliphora reared on this medium obtained some of their food requirements by digesting the cells of the gelatin-liquefying bacteria which are unavoidably ingested by the larvae. As the flies were all undersized, it appears that this microörganism is at least not a complete food for the insect (as yeast is in the case of Drosophila). Therefore, Bogdanow's conclusion, that bacteria play merely the part of liquefiers in the ecology of the larvae, is largely warranted.

Wollman ('11) repeated Bogdanow's experiments and came to the conclusion that sterile Calliphora vomitora larvae can be reared on sterile meat which has been sterilized by Tyndalizing rather than autoclaving. Bogdanow had autoclaved the meat used in his experiments, thus coagulating the proteins and making them insoluble to the larvae which when small have (according to Wollman) low proteolytic power. Though Wollman found that the larvae grew more successfully in the absence of microorganisms, as putrefactive bacteria always occur in the habitat of the fly, it is quite likely that they have some food value for the insect. Nevertheless, Calliphora, unlike Drosophila or Lucilia, grows best in the absence of all microörganisms.

ODORS ATTRACTIVE TO INSECTS

The odors which are attractive to dipterous adults are usually fermentation or decomposition products of the activity of micro-

organisms on a substratum. If we assume that the insect larva feed upon these microörganisms, the chain of circumstantial evidence is complete. Our knowledge of the odorous substance attractive to flies has been advanced greatly by the work of Barrows ('07) and Richardson ('16a, b; '17), but these authors hav never given the above interpretation to the response. Barrow studied the odors to which the adults Drosophila ampelophil (melanogaster) responds and found that the most attractive odor are those of ethyl and amyl alcohol, acetic acid, lactic acid, an acetic ether. A small amount of acetic ether, isobutyl acetat methyl acetate, acetic or butyric acid added to ethyl alcoh greatly increased its attractiveness. "Alcohol and acetic aci are commonly found in cider vinegar, fermented cider, and Cal fornia sherry in per cents that are close to those which call fort the largest number of reactions in Drosophila." This odor identical to that produced by the wine yeast Saccharomyce ellipsoideus which I have found to cause females to deposit t largest number of eggs.

In 1916 (a) Richardson reported that he had carried on a seri of experiments with odorous substances as baits for houseflie The baits tested were placed under wire-gauze traps and were following: Ammonium carbonate, ammonium sulphide sol tion, ammonium hydroxide, ethyl alcohol solution of skatol a indol, ethyl alcohol, acetic, formic, butyric and valerianic aci hydrogen sulphide solution and carbon dioxide. "Negative sults were obtained in all but the ammonium hydroxide and a monium carbonate experiments." The ammonia was the attr tive substance especially to females, which were found in percentage of 89.2 to 7.5 of the males, although the actual p centage of sexes in the vicinity was 54 to 45.9 respective Valerianic and butyric acid augmented oviposition; the femal however, showed some discrimination between nutritious non-nutritious material. In 1916 (b) a second paper gave a of insects attracted to the ammonia, all of which spend at le part of their life in some form of animal excrement.[27]

[27] A third paper ('17) showed that aqueous solutions of carbohydrates are less attractive than alcoholic or acetic acid solutions of such substances.

As both Drosophila and Musca domestica feed on microörganisms, it is of peculiar interest that the odors which stimulate oviposition by the female are identical to those formed by microorganisms in the substratum in which the insect normally breeds. The response of the female fruit fly to the odors of alcohol and acetic acid would indicate an instinctive response to the conditions best adapted to larval life, i.e., active fermentation. In the same way the response of the female housefly would indicate that the best conditions for housefly larvae require the presence of proteolytic (hence odors of ammonia, etc.) and fermentative (hence odor of alcohol and acetic acid) microörganisms. Richardson's results therefore give circumstantial support to my conclusion that the larvae of Musca domestica live on microörganisms.

Response to the odor of microörganisms is highly developed in the larvae of the yellow-fever mosquito, Stegomyia fasciata, as Bacot ('17) has recently shown that the eggs of this insect will remain for several months unhatched with the fully developed larva inside if the bacterial content of the surrounding water is low. The addition of foul contaminated fluid causes hatching in ten minutes. It is true that a fall of 6 to 10°F. causes some larvae to emerge, but the percentage is very low. Eggs were sterilized and transferred to sterile fluid, but if living yeast or bacteria were added they hatched immediately. Sterile autolized extract of brewer's yeast had the same effect, but killed bacterial cultures or watery extracts of yeast were ineffective. Bacillus coli was always effective when alive. The acidity and alkalinity of the different solutions were controlled. The author attributed the phenomena to the sense of smell of the larva and gave an exhibition of larvae feeding on stained bacteria (p. 178).

A rapid succession of different fungi occurs on manure (Gloyer and Fulton, '16, p. 6) and on other decaying substances together with an accompanying variety of odorous by-products. This succession determines the regular order in which decaying animal bodies become infested with insect scavengers. Mégnin ('85) and Hough ('97) have found that the order in which insects attack a decaying body is so constant that they have been able to develop a table giving the sequence of the different species. Thus

there are three stages of putrefaction and a final stage in which the dried tissues are consumed. The first stage of putrefaction is divided into two parts, viz., 'Body still fresh;' fauna consists of Musca domestica, Calliphora, etc.; 'Putrid odor develops;' fauna consists of Calliphora, Lucilia, Sarcophaga, etc. The workers of the second stage of putrefaction when butyric fermentation is taking place are Dermestes, Necrobia, Anthomyia, etc. The third stage, the stage of ammoniacal fermentation, is accompanied by infestation with such forms as Silpha, Necrophorus, Hister, Aphyra, Phora, and many Acarina. Finally the dried tissues are consumed by Aglossa, Tinea, Anthrenus, etc., and the bodies of these are destroyed Ptinus.

As this succession of species in the fauna of dead bodies holds fairly constant, it seems plausible that the odors produced are the determining factor and that the microörganisms producing the odors are of great importance to the insect as food and as solvent agents. Therefore we may conclude that the odors of fermentation and putrefaction are attractive to insects because they indicate a substratum made suitable for the insect by the abundance or the action of microörganisms.

MICROÖRGANISMS AS FOOD OF OTHER ANIMALS

The use of microphytes as food is not confined to insects and mites alone, but is quite common among Protozoa. The effect of pure culture of different species of bacteria on Paramecium has recently been described by Hargitt and Walter ('16). These authors were able to sterilize the animal by six successive washings in sterile water and then raised them on pure cultures of thirty different species of bacteria. They found that the bacteria from fresh were more favorable than those from older infusions.

It is probable that many of the Nematoda are also mycetophags. Anguillula aceti, the vinegar eel, which inhabits the 'mother of vinegar' and is also found in sour flour paste, and many of the 'parasitic' nematodes found in decaying plant tissue may be attracted at first by exposed soft tissue and later feed upon the microörganisms in the decay with which they infest the plant.

Since Darwin's work ('81) it has been assumed that the earthworm finds its food in the humus of the soil it infests. Humus soil is notably rich in microörganisms, for these are the elaborators of humus from plant and animal remains, and it is possible that they are of food value to the worm.

Such structures as the endostyle and dorsal pharyngeal groove of Amphioxus and the Tunicates are probably for the purpose of entrapping microphytes of various kinds.

As pointed out by Osborne and Mendel ('14 b), microörganisms may also be of value to higher animals as elaborators of protein in the digestive tract from the non-protein substances ingested. This would be especially true in herbivorous animals, as Armsby ('11) has shown that non-protein substances are a source of protein in these animals, probably due to the formation of digestible bacterial protein in the digestive tract. The possibility that the flora of the digestive tract may modify the food elements supplied in a nutritional experiment is a drawback to the use of mammals in such experiments. An insect like Drosophila should be of value as material for such experiments because of the ease with which it is sterilized.

Many attempts have been made to rear mammals under sterile conditions, but most of these have failed so that it has been a great question whether or not it is possible for animals to live in the complete absence of microörganisms. As a large flora normally occurs in the digestive tract, it was necessary to sterilize the animal before it had taken food and to keep it in a sterile environment, therefore Pasteur ('85) suggested the use of hens, the eggs being well fitted for sterilization. Pasteur's suggestion was later carried out, but the first experiments were made by Nuttall and Thierfelder ('95–'96) on guinea-pigs, the young being removed aseptically from the mother by cesarian section. The animals were kept in a complicated aseptic environment and were fed upon food of animal origin. The animals gained 10 grams in one week (84 grams total weight) and appeared to be normal on the eighth day, when the experiment had to be discontinued.

In 1896 to 1897 the experiment was repeated, the gain in weight in eleven to thirteen days was almost normal, being 108 to 132 instead of 130 to 180 grams. In a third paper ('97) the authors experimented with the hen's egg and found that it was not sterile; they also summed up their previous work in the conclusion that animals just born do not grow well in the absence of microörganisms. Schottelius interprets the increase in the weight due of guinea-pigs in these experiments as due to the coagulation of caseinous material, from the milk, on the lining of the digestive tract. This author used hen's eggs as material to sterilize, but after a brilliant series of experiments ('99, '02, '08) has arrived at the conclusion that normal life without bacteria is impossible, as all the sterile individuals reared are retarded and stunted. Mme. Metchnikoff ('01) and Moro ('05) obtained similar results with tadpoles. In 1908 Tibbert, from theoretical considerations, came to the conclusion that higher organisms cannot live in the absence of microörganisms because each species of animal harbors definite numbers and species of bacteria. Metchnikoff, Weinberg, Pozerski, Distaso, and Berthelot ('09), on the other hand, reared the fruit bat Pteropus medius to normal size under practically aseptic conditions, and Cohendy ('12) kept chicks alive in anaseptic condition from twelve to forty days. Cohendy's conclusions are as follows:

> La vie sans microbe est possible pour un vertébré—le poulet—pourvu normalement d'une riche flore microbienne.
>
> Cette vie aseptique n'entraîne par elle-même aucune déchéance de l'organisme.

Kianigin ('17) has recently reopened this question by a review of all the literature. At first sight it appears incomprehensible that aseptic life should be so difficult when the greatest quantity of microörganisms is located in the non-digestive portions of the digestive tract. Metchnikoff ('09) points out, however, that the digestive powers of newly born are much weaker than those of older animals. The increasing number of cases of organisms which can be raised aseptically indicates that an aseptic existence may be possible in the majority of cases.

Nencki ('86) gave indirect evidence in this direction when he showed that the action of digestive ferments on food-stuffs makes them very quickly soluble and absorbable. The action of bacteria merely carries the decomposition to a lower level, yielding unassimilable aromatic acids, fatty acids, phenol, kresol, indol, skatol, carbon dioxide, methan, etc. The indications are, however, that microörganisms are of value as intestinal flora not because of their digestive, but because of their synthetic power.

MICROÖRGANISMS AS INTERNAL SYMBIONTS OF INSECTS

In addition to the cases of internal symbiosis of fungi with insects inhabiting dry wood, many Hemiptera and Blattidæ are also associated with microörganisms. These are usually bacteria or yeasts and infest the ovary. For example, the pseudovitellus of the aphid was long a puzzle to embryologists, but finally proved to be a granular body containing yeast cells which in the further development of the insect make complicated migrations and finally become lodged in certain fat-bodies which are termed mycetocytes or bacteriocytes, after which the infection of the ovary takes place. The evidence that a real symbiotic relationship exists in the Blattidæ and Hemiptera is given by Buchner and others as follows:

1. All eggs are infected.
2. The infection is not injurious to them.
3. Each species of insect is associated with a definite species of microörganism.
4. This association is very definite and almost a specific character.
5. The yeasts profit by the relationship in the protection which they receive from the host against the vicissitudes of the environment.
6. The yeasts multiply as the animal multiplies, always being present in constant amount even in such rapidly increasing forms as aphids and coccids.
7. The yeasts and bacteria are of value to the host as destroyers of waste products of metabolism, such as urates, according to Sulc ('10b), and as absorbers of excess food materials such as sugar, according to Pierantoni ('10).

The movements and location of the symbionts has been studied by Buchner ('12) in a great work on the Blattidæ. Glasgow ('14) has also studied a case of symbiosis in which the microörganism, instead of being located in a fat-body (mycetocytes), as in the cockroaches, is retained in very highly developed gastric caeca of the plant bugs (Heteroptera). The function ascribed to the bacteria by Glasgow is the prevention of infection of the digestive tract by other bacteria. Dissected digestive tracts on bacterial media gave only pure growths of the associated microörganisms. Petri ('04, '05, '06) studied the similar case of Dacus oleae, which feeds on the olive, but ascribes to the bacillus alipolytic enzyme of assistance in the digestion of the food. Schaudinn ('04) finds that a fungus (Entomophthorineae) is transmitted through the egg of Culex and is always found in the diverticulae of the oesophagus of adult Anopheles and Culex. He has been able to rear the fungus in sugar solution and has demonstrated that CO_2 is formed in the imago from the sugars in the blood which it has sucked up. It is probable that the irritation caused by the bite of mosquitoes is largely due to enzymes secreted by these fungi.

In these cases the associated fungus may be a commensal, in its relation to the host, profiting by an oversupply of some food substance, as in the case of aphids, mosquitoes, etc., or may be of value as a chemical agent, as in the case of Dacus oleae, or may be of service in maintaining an unchanged digestive flora, as in the Heteroptera, as described by Glasgow. In general, however, the exact function of the microörganism to its host has not been thoroughly explained.

CONCLUSION

I have shown by experiments that Drosophila living in fermenting fruit are dependent for their food supply on the synthetic and absorptive powers of yeast cells. In a similar manner, my study of the relation of Musca domestica to manure, of Desmometopa to decaying meat, and of Sciara and Tyroglyphus to decaying wood shows clearly that these Arthropods also feed on microörganisms. I have also endeavored to account for the origin and devolopment of this habit, to ascertain the probable extent of its occurrence, and to consider the known associations of animals with fungi in general. The experiments and considerations all tend to establish the principle that insects inhabiting fermenting and decaying substrata of low protein content, usually feed upon the microörganisms present and thus benefit by the power of the fungi to extract, adsorb, and synthesize many non-protein nitrogenous compounds.

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