Formation of Associations in the May-fly Nymphs *Heptagenia Interpunctata* (Say)

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Two figures

PROBLEM

While working on the life history and general behavior of *H. interpunctata*, I observed, on several occasions, what appeared to be indications of learning. These cases suggested

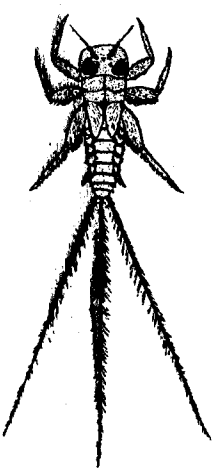

FIGURE 1. May-fly nymph

several forms of experiments. Three different ways of testing the learning capacity of the nymphs were devised and carried out, namely, experiments on reactions to objects in their thigmotactic relations, experiments on fear reactions as results of associations with pain, and experiments on the formation of associations in feeding. A considerable amount of work has been done on the learning capacity of higher insects, but the

formation of associations in lower insects has hitherto been the subject of very few experimental investigations.

REACTIONS TO OBJECTS

Different nymphs vary greatly in their response to objects under similar conditions. Some, for instance, will go as far as 11 inches in a direct line toward a stone about one cubic inch in size, while others seem to be unaware of the object even when it is much nearer.

I experimented with 20 specimens in a large glass basin, evenly lighted and evenly colored. The nymphs were placed,

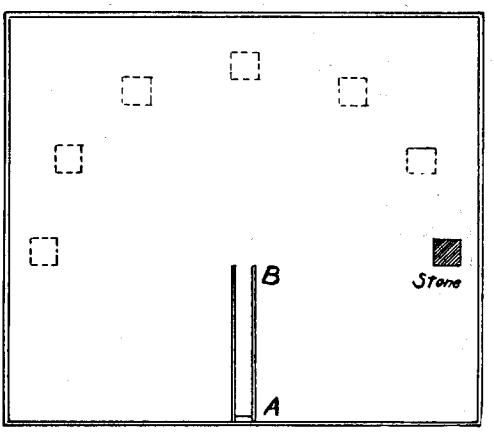

FIGURE 2. Diagram of apparatus used in the experiments on reactions to objects

one at a time, in a small glass passage, represented by AB in the diagram, within and close to one side of the large basin, and a stone a little more than one cubic inch in size was placed about a foot away from the opening of the glass tunnel.

The little glass tunnel was to prevent random movements. Specimens dropped into water, owing to excitement, are apt to swim about in any direction. A specimen would be carefully placed at the closed end of the passage, which was sufficiently wide to enable it to move freely forward, but which would not permit it to turn around. Slight stimulation from behind induced it to swim forward. In doing so it had ample time to get a view of the enclosure before it reached the open

end of the tunnel. If the nymph, after emerging from the tunnel, did not go in a straight line in the direction of the stone, the stone would be brought closer at each trial until a distance was obtained at which the insect would make several successive straight trips to it. The stone would then be placed at the same distance, but at various points in a semi-circle in front of the opening, at different times. Table I shows the variations in the distance at which the first definite trip was made by each one of the different individuals experimented with, and also the percentage of straight trips made by each individual toward the stone, at the recorded distance, in 10 successive trials.

TABLE I

INDIVIDUAL VARIATIONS AMONG H. INTERPUNCTATA IN REACTIONS TO OBJECTS IN AN EVENLY ILLUMINATED ENVIRONMENT

Specimen	Distance in inches	Percentage of straight trips
1	11	70
2	9.5	90
3	9	90
4	8.5	100
5	7	80
6	7	70
7	6.5	100
8	7	80
9	6	90
10	6	80
11	6	80
12	6	70
13	5.5	100
14	5.5	80
15	5	90
16	5	70
17	4	100
18	4	80
19	3.5	100
20	3	70

In another experiment on variations the same apparatus was used, but the source of light was a 16 candle-power incandescent lamp attached to one end of the basin. In order to reach the stone the nymphs were obliged to swim directly toward the light.[1]

[1]Wodsedalek, J. E. Phototactic reactions and their reversal in the May-fly nymphs *H. interpunctata* (Say). Biological Bulletin, 1911. vol. XXI., pp. 265–272.

Normally the nymph is decidedly negative in its phototactic response. Fifteen specimens were used, one after another, beginning with the stone one inch away from the opening of the small glass tunnel, on the side toward the light, and then increasing the distance until all of the nymphs reacted negatively.

Here again, we have considerable variation, as is shown in table II. For example, all of the 15 specimens went toward the stone, against the light, when it was between one and two inches away from the opening of the tunnel, and when it was between three and four inches away only about half of them went toward it, while, when the stone was placed six inches away, all of the specimens went in the opposite direction.

The uniformity manifested by the nymphs in their negative response to light when no objects are present, and the obvious variations evinced in their reactions to objects in an evenly lighted environment and also in their reactions to objects against the rays of light, seem to suggest that the specimens going the greatest distance toward the objects have learned to do so through experience in their natural environment.

TABLE II

VARIATIONS AMONG NYMPHS IN THEIR RESPONSE TO OBJECTS AGAINST THE RAYS OF LIGHT

Distance	Toward stone, against light	Away from the light
1 inch	15 trips	0 trips
1.5 "	15 "	0 "
2 "	14 "	1 "
2.5 "	13 "	2 "
3 "	11 "	4 "
3.5 "	9 "	6 "
4 "	3 "	12 "
4.5 "	1 "	14 "
5 "	1 "	14 "
5.5 "	1 "	14 "
6 "	0 "	15 "
6.5 "	0 "	15 "

In order to ascertain whether the reactions to objects are modified by experience, three of the nymphs having similar

records in going toward a stone in an evenly lighted environment were taken and with each 21 daily experiments were performed. At the beginning of each set of trials the stone was placed at the greatest distance at which the nymphs went toward it on the preceding day. Usually, the stone had to be brought somewhat closer than this before the nymph made its first trip toward it. Then the stone was taken farther away after each successful trip, until a distance was reached when the nymph seemed to pay no attention to the object. This necessarily had to occur during each set of trials in order to ascertain the greatest distance at which the nymphs would go to the stone. An over-rating of the distance necessitated the bringing back of the stone to a place where another successful trip was made. The distance made each day on the twelfth successful trip was recorded. The results of this test of the learning ability of three individuals are given in table III. The number of inches indicate the distance each nymph would go toward the stone at the end of 12 successful trials. It will be noticed that two of the specimens increased their distance slowly but steadily, while one varied in its daily behavior and even at the end of three weeks apparently had learned nothing.

TABLE III

SHOWING THE LEARNING CAPACITY OF THREE *H. Interpunctata* NYMPHS

Date	Number I	Number II	Number III
1	7 inches	6.5 inches	6.5 inches
2	7 "	7.5 "	8 "
3	7.5 "	7.5 "	8. "
4	8 "	8 "	5.5 "
5	8 "	8 "	7 "
6	7.5 "	8.5 "	8.5 "
7	8 "	8 "	6 "
8	8 "	8.5 "	8 "
9	8.5 "	9 "	7.5 "
10	8.5 "	9 "	5 "
11	8.5 "	9.5 "	8 "
12	9 "	10 "	7.5 "
13	9 "	9.5 "	8 "
14	8.5 "	10 "	8 "
15	9 "	10 "	5 "
16	10 "	11 "	7.5 "
17	9.5 "	10 "	6 "
18	10 "	11 "	8 "
19	10 "	19.5 "	6 "
20	10 "	11 "	7 "

Then 10 specimens that had poor records in going toward a stone against the light were taken and 21 daily experiments were performed with each. The apparatus of the foregoing experiments was used, except that the 16 candle-power incandescent lamp was again introduced at one end of the basin and the specimens, in order to swim toward the stone, had to go directly toward the light. The method of procedure was practically the same as that of the previous investigations. The results were surprising, for most of the specimens gradually inhibited their normal negative phototaxis and proportionately reinforced their positive reaction to the stone. However, the individuals varied considerably. Some showed a slow but steady progress for almost three weeks; some were quite docile for a few days, but soon reached a limit; others learned very little; still others would go toward the stone a fair distance at the end of each set of 21 trials, but had to be started at almost the same distance each day. The four different classes of behavior are represented in table IV.

TABLE IV

SHOWING FOUR DIFFERENT CASES OF LEARNING TO SWIM TOWARD OBJECTS AGAINST THE RAYS OF LIGHT

DAY	SPECIMEN I		SPECIMEN II		SPECIMEN III		SPECIMEN IV	
	First Trip	Last Trip	First Trip	Last Trip	First Trip	Last Trip	First Trip	Last Trip
1.....	1.5 in.	3 in.	1.5 in.	4 in.	1 in.	2 in.	1 in.	4 in.
2.....	2 "	3 "	2.5 "	5.5 "	1 "	1.7 "	1 "	4.5 "
3.....	1.7 "	3.5 "	2.5 "	6 "	1 "	2.5 "	1 "	5.5 "
4.....	2 "	4 "	3 "	6 "	1.2 "	3 "	1.2 "	6.5 "
5.....	2.5 "	5 "	3.5 "	6.5 "	1.5 "	3 "	2 "	7 "
6.....	2.5 "	4.2 "	3 "	7 "	1.2 "	2.5 "	1.5 "	7 "
7.....	2.7 "	5 "	4 "	7 "	1 "	3 "	1 "	6.5 "
8.....	3 "	5 "	4 "	7.7 "	1 "	3.5 "	1.2 "	7.5 "
9.....	3 "	5.5 "	3.5 "	8 "	1.5 "	2.5 "	1.7 "	8 "
10.....	3 "	5.7 "	4 "	7.2 "	1.5 "	2.7 "	2.5 "	7.7 "
11.....	2.7 "	6 "	3.7 "	8 "	2 "	2.5 "	1.5 "	7 "
12.....	3.7 "	6 "	4 "	8 "	2 "	3 "	1 "	6.5 "
13.....	3.2 "	6 "	4.5 "	8.2 "	2 "	2.7 "	3.7 "	8 "
14.....	3 "	6.2 "	5 "	8 "	1.7 "	3.5 "	2 "	7.2 "
15.....	3.5 "	6.5 "	4.5 "	8 "	1.7 "	3.2 "	1 "	6 "
16.....	3.5 "	6.5 "	4. "	8 "	2 "	3 "	3 "	8.5 "
17.....	4 "	6.5 "	5.2 "	8.5 "	2.5 "	3 "	2.5 "	7.7 "
18.....	3.5 "	7 "	5.5 "	8 "	2.7 "	3.7 "	1.7 "	8 "
19.....	3.7 "	8 "	4 "	8 "	1.7 "	3.5 "	1 "	7.7 "
20.....	4 "	7.5 "	5.5 "	8.5 "	2 "	2.5 "	2.5 "	8.5 "
21.....	4 "	8 "	5.7 "	8 "	2 "	3.5 "	1.7 "	7.5 "

Here again, at the beginning of each set of trials the stone was placed at the greatest distance at which the specimen went toward it on the preceding day, and then if necessary, it was brought closer until the nymph made a straight trip toward it, after which the distance was again gradually increased. The number of inches in the first column shows the distance at which the nymph made its first trip toward the stone each day, and that in the second column indicates the distance at which the trip was made at the end of 15 successful trials.

The experiments demonstrate the learning capacity of *H. interpunctata*. Not only did the insect show a tendency to learn in an environment free from disturbing factors, but, as can be seen from the results of the last experiments it learned to do greater and greater distances toward a stone against the rays of light. This is a fact of considerable interest, since these nymphs have a decided negative phototaxis. But, on the other hand they are very positive in their thigmotactic response, and the results seem to indicate beyond doubt that the satisfaction derived through attachment to the stone counteracted whatever reluctance the nymph may have experienced in going against the light.

FEAR REACTIONS

For this set of experiments I first took two large dishes and placed six vigorous specimens in each. Those in one dish were disturbed several times each day, while those in the other were left unmolested. Although the insects always show a tendency to gather into groups when placed in a dish of water, the tendency is greatly diminished in a few days. Such was the case in the undisturbed group. In the group undergoing disturbance this tendency was greatly augmented, apparently through fear. To overcome the grouping propensity the different individuals were put in separate dishes with particles of food too small to enable the insects to attach themselves, and to further obviate the discomfort occasioned through the lack of proper thigmotactic stimuli, the smooth glass bottoms of the dishes were covered with sand.

The room for this research was free from disturbance, and before investigations for the procuring of detailed data were attempted careful observations were made on the behavior of a set of nymphs with a view to detecting and overcoming any

undesirable features of the environment. Then, six specimens in separate dishes were placed at one end of the room and subjected to four daily disturbances. In another part of the room were three individuals undergoing one disturbance each day; and some distance away were three more, disturbed but once in three days. In separate dishes scattered among these were six other individuals undergoing no disturbance whatever. Careful observations were made and detailed records of the behavior of all the specimens were kept.

Tables V and VI suffice briefly to show the results obtained with two of the specimens. Their behavior was characteristic of more than half of the 18 nymphs observed in this manner. The letters A, B, C, and D, designate the behavior displayed on the first, second, third, and fourth daily observations respectively. Each one of the three columns is intended for a particular form of behavior displayed by the nymphs, and the letters indicating the behavior manifested during the four daily observations are placed in the columns accordingly. Omission of a letter means that the nymph was not easily disturbed on that occasion. In the first column are recorded the cases of indications of fear only when the specimen was slightly disturbed with a dissecting needle. In column II are given the cases of signs of agitation when a hand was passed back and forth above the dish, and in the last column are indicated the signs of fear displayed by the specimen in the way of swimming about in an excited manner when I appeared before the enclosure. For example, on March 31, on the first observation, which was in the morning, specimen number one showed signs of fear only when the water near it was disturbed with a dissecting needle, and hence (A) was placed in the first column. On the second observation, which was at noon, the nymph began to swim about in an agitated manner when my hand was moved back and forth over the dish, thereby making a place for (B) in column two. On the third observation, which was about six o'clock in the afternoon, the insect displayed indications of fear when I appeared before the enclosure, and so (C) naturally fell in column three. On the last observation on that day, which was late at night, the nymph again showed signs of fear when a hand was moved back and forth over the dish, which caused (D) also to be in the second column.

A glance at either table V or VI at once suggests a curve representing the obvious intensification of the expression of fear. The letters indicative of signs of fear first appear in great numbers in the first column and later gradually make their appearance in columns two and three which represent the more obvious signs of agitation. All of the specimens, however, did not behave in this manner. Some, from the very beginning, would feign death when disturbed, while others reacted very favorably for several weeks and then, on account of their death-feigning propensity, which became more and more pronounced, little could be done with them.

TABLE V

FEAR REACTIONS IN *H. Interpunctata*, WHEN DISTURBED FOUR TIMES EACH DAY
SPECIMEN I

DATE	Indications of fear only when specimen was slightly disturbed with dissecting needle	Manifest signs of agitation when hand was passed back and forth above the dish	Signs of fear displayed in the way of swimming about in an excited manner when I appeared before the enclosure
March 20	C		
21	A C D		
22	B D		
23	A B C D		
24	A C D	B	
25	B D	A	
26	B C	A D	
27	B D	A	
28	A D	B C	
29	A B	C D	
30	B C	D	
31	A	B D	C
April 1	D	A C	B
2	B	A	C D
3	C	D	B
4		B C	A D
5	B		A C D
6		A D	B C
7		C	A B D
8		D	A B C
9	A	B	C D
10		B	A C D
11		B	A B C D
12		D	A B C

TABLE VI

FEAR REACTIONS IN *H. Interpunctata*, WHEN DISTURBED FOUR TIMES EACH DAY
SPECIMEN II

DATE	Indications of fear only when specimen was slightly disturbed with dissecting needle	Manifest signs of agitation when hand was passed back and forth above the dish	Signs of fear displayed in the way of swimming about in an excited manner when I appeared before the enclosure
March 20	B C		
21	A C		
22	A C D		
23	A B C D		
24	A B C D		
25	A C D		
26	A B D	C	
27	C D	A	B
28	A C D	B	
29	A B C D		
30	A B C D		
31	B C D	D	
April 1	A C D	B	
2	B C D		A
3	A B C D		
4	B D	A C	
5	B C D	A	
6	A B	C D	
7	A	B C D	
8	C	A D	B
9	A D	B C	
10	B	A D	C
11		B C D	A
12		A B C D	
13	B	A	C D
14	C	B D	A
15		A B C D	
16		A C D	B
17	D	B	A C
18	B	A C	D
19			A B C D
20		A B	C D
21		B	A C D
22		A D	B C
23		C	A B D
24	C	A	B D
25		B D	A C
26		D	A B C
27		A	B C D
28	A	C	B D

In table VII are given the results obtained with three of the individuals which were observed and disturbed once each day. In this case the numbers 1, 2, and 3, represent the three speci-

mens, and the position of the numbers in any of the columns, like the position of the letters in the two previous tables, indicates the particular kind of behavior displayed by the specimen on that day. For example, on April 13, specimens one and two had to be slightly disturbed before they manifested signs of fear, while specimen number three on the same day showed signs of fear when I appeared before the dish.

TABLE VII

FEAR REACTIONS IN THREE SPECIMENS OF *H. Interpunctata*, WHEN DISTURBED ONCE EACH DAY

DATE	Indications of fear only when specimen was slightly disturbed with dissecting needle			Manifest signs of agitation when hand was passed back and forth above the dish			Signs of fear displayed in the way of swimming about in an excited manner when I appeared before the enclosure		
1	1	2	3						
2	1	2	3						
3	1	2	3						
4	1	2	3						
5	1		3		2				
6	1	2	3						
7		2	3						
8	1	2							
9	1	2	3						
10	1	2	3						
11		2	3						
12		2	3						
13	1	2							3
14	1	2	3						
15	1	2							
16	1		3						
17	1	2	3						
18		2	3	1					
19	1	2	3						
20		2	3	1					
21	1	2							
22	1	2	3						
23	1	2	3						
24	1	2	3						
25		2	3						
26	1	2	3						
27	1	2				3			
28		2	3						
29		2				3			
30	1		3					2	

These results seem to indicate that the nymphs formed few, if any, associations with pain resulting from a single daily disturbance. For the specimens disturbed once in three days there was even less evidence. Their behavior was almost identical with that of the undisturbed group, of which, during two months, one manifested signs of fear only twice; another three times; and four never stirred when I appeared or moved my hand over the dish. In the forms disturbed several times each day, however, even after making liberal allowance for accidental movements, there remains abundant evidence that the nymphs learned to associate my presence with discomfort.

FORMATION OF ASSOCIATIONS IN FEEDING

Training nymphs to come for food is a tedious task. This is due to many reasons, the most obvious of which appear to be: (1) The fact that in their natural environment they always feed attached to the under side of stones with their dorsal side downward; (2) they are moderate feeders and can go a long time—as long as two months—without food; (3) they are strong in their thigmotactic response, and great care must be exercised in dealing with the specimens in order to avoid fear reactions.

Six active specimens were selected and placed in separate dishes without food, in a compartment of the room employed in the experiments on fear. Daily observations were made as regards the position of the nymphs, and considerable time was spent each day in attempting to induce the different individuals to follow food to the end of the dish nearest to me.

At first a piece of alga was brought near the antennae of the nymphs with a pair of forceps. Within a few days practically all of the individuals ate the offered particle. A number of trials showed that only a small morsel of food was necessary each day to insure the proper response the succeeding day. As the nymphs would cling to the piece of alga with their fore claws I would hold on to the food with the forceps and turn the nymph so that it always faced me. This had to be done with considerable care in order not to dislodge the insect or interfere with its feeding. Then I would pull on the piece of food, endeavoring to induce the nymphs to follow. At first they would not move any great distance, but would plant themselves firmly and, if the pull was continued, the particle of

food would either break or the nymphs would make an attempt to attach themselves to the forceps.

Later, I would hold a piece of alga near the head of the nymph and when it made an attempt to secure the food I would withdraw a little, in which case the insect would slowly crawl after it. This was a difficult task, for if the food was too far away the nymph would make no attempt to secure it, and again, when it was brought nearer, the insect would make a quick snap at it before I had time to withdraw it. For several weeks I was not able to entice the nymphs to follow the food any considerable distance before they would have their claws on it. However, when this was done the food was not taken away, but, by means of a gentle pull the insect was brought to the part of the dish nearest to me where it was allowed to feed, to a limited extent, undisturbed.

After experimenting with the insects in this manner daily for about four weeks I noticed that most of the specimens would frequently swim after the food when it was brought near them, and would often swim toward me when they happened to be at the further end of the dish when I made my appearance or moved my hand over them. Not all of the individuals which I experimented with reacted so favorably. Some, on account of their decidedly strong thigmotactic propensity, proved to be entirely intractable; while others absolutely refused to eat, and swam about very much agitated whenever a particle of food was brought near them. However, after two and a half months a large majority of the specimens plainly manifested, by means of swimming toward me or clawing against the side of the dish nearest to me, that my presence meant food to them.

From table VIII, on the feeding reactions of three of the specimens, it will be seen that not only did the insects show a growing tendency to remain in the feeding portion of their field, but that they learned to swim after the food. In column I, opposite the number of the day of the experiment, are indicated the positions of the three nymphs A, B, and C, respectively. The (+) sign indicates that the nymph occupied the end of the dish at which I always made my appearance and where the nymph was allowed to feed; the (—) sign indicates that the specimen was at the opposite end. For example, at the time of the observation on the third day of the experiment, specimen A was at the (+)

end of the dish or the end nearest to me, and specimens B and C were both at the (—) end, or the end farthest away from me.

Columns II, III, and IV show the gradual formation of associations in the three specimens. For instance, in column II are given the names of the specimens which have eaten some offered food, but would not follow it on the day opposite which the names are set; column III represents the cases of the same specimens when they followed the food as it was held near their mouth-parts with a pair of forceps; and in the last column are indicated the names of the same specimens which, when present in the (—) end of the dish would swim to the (+) end for food when I appeared or moved my hand over the dish, and the letters A, B, and C, which represent the three specimens, are placed in any one of the last three columns according to the kind of behavior manifested during the different daily observations. For example, on the fourth day of the experiment letters A, B, and C, occur in the second column which means that on that day all three of the specimens ate an offered particle of food, but would not follow it; nor did specimens B and C which were at the (—) end of the dish, swim to the (+) end when I appeared before them. On the twentieth day specimen A, which was at the (+) end of the dish, ate some food but did not care to follow it; specimen B, which was at the (—) end of the dish followed the food when it was held near it with a pair of forceps and hence B is placed in column III; and specimen C on the same date was also at the (—) end of the dish, but it swam toward the (+) end when I appeared and hence C was placed in column IV which is intended for that type of reaction.

It will be seen from the table that all three of the specimens indulged in food within a few days, but that not until the end of three weeks did all three swim after the food when it was offered to them with a pair of forceps. The most obvious signs of the formation of associations, recorded in the last column, did not appear until even later. The number of cases showing that form of behavior are noticeably less than those in the preceding columns, but it must be remembered that during the last days of the experiment the insects occupied the (+) end of their compartment the greater part of the time. Very frequently when the nymphs were at the (+) end at the time

of my presence, the ends of the dish would be reversed, causing the specimen to be at the end farther away from me. This action was scarcely complete when the watchful nymphs would again be facing me and immediately proceeding on a rush to regain their former relative position. Such cases, however, are not recorded in the table.

TABLE VIII

SHOWING GRADUAL FORMATION OF ASSOCIATIONS IN THREE SPECIMENS OF *H. Interpunctata* IN THEIR FEEDING REACTIONS

Date	COLUMN I: Position of the nymphs in the dish. (+) indicates the end of the dish at which I always made my appearance and where the nymphs were allowed to feed; (—) indicates that the nymph was at the opposite end						COLUMN II: Indications of the cases in which the nymphs have eaten an offered particle of food, but would not follow it			COLUMN III: Record of the cases when the nymphs would follow a piece of food when it was held near their mouth parts			COLUMN IV: Record of the cases in which when the specimen was present at the (—) end of the dish it would swim to the (+) end when I appeared or moved a hand over it		
	Specimens														
	A		B		C										
1		—	+			—			C						
2		—		—	+			B							
3	+			—		—	A								
4	+			—		—	A	B	C						
5		—	+		+		A					C			
6		—	+		+		A								
7	+			—		—		B							C
8	+		+			—	A	B							
9		—	+		+							C			
10	+			—		—	A								
11		—		—		—	A				B				
12	+		+			—									
13	+		+		+		A	B	C						
14	+			—		—	A	B				C			
15		—		—		—	A								C
16		—	+		+										
17	+			—	+		A	B				C			
18		—		—		—									
19		—	+		+						B	C			
20	+			—		—	A				B				C
21	+			—	+		A								
22		—	+		+				C	A	B				
23		—		—		—	A							B	C
24	+		+		+		A				B	C			
25		—	+		+			B		A					
26		—	+			—				A	B	C			
27	+			—		—				A	B				C
28		—	+		+					A	B				
29	+			—		—			C				A		
30		—	+		+		A				B				

TABLE VIII—*Continued*

Date	Column I: Position of the nymphs in the dish. (+) indicates the end of the dish at which I always made my appearance and where the nymphs were allowed to feed; (—) indicates that the nymph was at the opposite end						Column II: Indications of the cases in which the nymphs have eaten an offered particle of food, but would not follow it			Column III: Record of the cases when the nymphs would follow a piece of food when it was held near their mouth parts			Column IV: Record of the cases in which when the specimen was present at the (—) end of the dish it would swim to the (+) end when I appeared or moved a hand over it		
	Specimens														
	A		B		C										
31		—	+			—					B	C	A		
32		—		—	+					A		C		B	
33	+		+		+						B				
34	+			—		—									C
35	+			—	+				C	A					
36		—		—	+							C		B	
37	+			—	+					A	B	C			
38	+		+			—					B	C			
39		—	+		+							C	A		
40	+			—	+					A		C		B	
41	+			—		—				A	B				C
42	+			—		—									C
43	+		+		+		A							B	
44		—	+		+				C		B		A		
45		—	+		+						B	C	A		
46	+			—	+					A		C		B	
47	+		+			—		B		A		C			
48		—	+		+					A		C			
49	+		+		+						B		A		
50	+			—		—								B	C
51	+		+		+							C			
52	+			—	+							C			
53	+		+		+				C	A					
54	+		+		+						B	C			
55	+		+		+						B				
56		—	+		+			B		A					
57	+		+		+							C	A		
58	+			—		—						C		B	
59		—	+		+						B		A		
60	+		+		+		A					C			
61	+		+		+					A				B	
62	+			—	+						B	C	A		
63		—	+		+								A		C
64		—	+			—				A				B	
65	+			—	+					A		C			
66		—	+		+						B				
67	+		+		+							C		B	
68	+			—	+					A					
69	+														
70	+	—											A		
71	+	—													
72	+														
Average	43	29	40	28	44	24									

Should a line be drawn through the parts of the table where the letters appear most abundantly we should have a gradual curve representing the reinforcement of the reactions to a given stimulus as a result of the formation of associations. The behavior of the three nymphs represented in the foregoing table is characteristic of many other nymphs experimented with. To check these results, daily observations were made in regard to the position and behavior of another set of individuals which had a food supply scattered in small particles at the bottoms of their dishes. Their records show that they exercised no choice between the two ends of the dish, and that they never swam toward me as did those in the training experiments. The trained nymphs no doubt formed an association.

Not infrequently during the work with *H. interpunctata* nymphs did I observe specimens which behaved in an uncommon manner. The most interesting individual which attracted my attention was in the midst of a large number of newly collected specimens in a dish of water. Its peculiar conduct led me to observe it carefully, when I noticed that it skipped about among its fellows, lashing its setae and occasionally making a pass at one of the other nymphs with its front limb like a playful kitten.

It was placed in a separate dish and taken to my room where it was observed and trained daily. After a few days I noticed that its tendency to remain on the upper surface of the stone, whenever the stone was inverted, became more and more pronounced. The nymph was brought to the upper surface in that manner several times each day and on every occasion it was offered a particle of alga. So long as it was treated gently it would remain in that position for a considerable time, but, the moment it was dealt with roughly it would conceal itself under the stone. Great care was taken not to frighten the insect, and within a few weeks it would remain on the upper surface of the rock for hours at a time facing me. Whenever I noticed that the nymph had gone under I would turn the rock over. In about two weeks more, it had almost completely inhibited its shyness and would follow a particle of food to any part of the stone. Then I removed the much coveted stone and placed in the dish a stone bearing a finger-like projection about three inches long. The stone was so submerged that the extreme

point of the process projected about half an inch above the surface of the water, pointing toward me. The insect at this stage would readily follow a piece of food to the projection, which became its regular feeding place. At the end of about two months the insect spent practically all of its time, during my presence, perched up near the point of the projection with its head just below the surface of the water. The time and clearness of the day seemed to play no important part on its behavior. Even at night when the electric light was directly over th table on which the insect was located, it would not desert its favorite resting place. Almost invariably, when I had left the room the nymph would soon retire to the under side of the stone. All that was necessary, at this time, to bring the specimen up when it had disappeared from sight was to slightly jar the dish or the table on which the dish was located, and the insect would quickly come up to the upper side of the rock and make directly for its feeding place. Later, very frequently as I stepped into the room I caught the insect in the act of coming out from its hiding-place and halting at the end of the projection ready to receive its usual reward.

Sometimes, when I had been gone from the room all day and did not feed the nymph shortly after my appearance, but simply moved my hand over it, the insect would slowly crawl up on the point of the rock extending out of the water. However, it usually exposed only its head and thorax to the air and apparently exercised considerable care not to expose the abdomen, which bears the tracheal gills. Although I anticipated extended experiments with this entertaining individual, all was brought to a close by its metamorphosis. Nothing more could be done with it in the adult stage, as the Ephemeridae never take food after they emerge, and live but a few days at most. Here again, we have obvious signs of the formation of associations.

Sondheim[1] accidentally made some observations and later performed some experiments with a dragon-fly larva, *Aeschna grandis L.*, and obtained practically similar results during a much shorter time and with far less effort. This is probably due to the fact that the natural habits of *A. grandis* are much

[1] Sondheim, Maria. Wahrnehmungsvermögen einer Libellenlarve. Biologisches Centralblatt, Bd. 21, S. 317-319. 1901.

different from those of *H. interpunctata.* However, she obtained such results with only a single specimen and although attempts were made to investigate the learning capacity of another larva of the same family, she was unable to get any further evidence of the formation of associations. " Seit einigen Wochen befindet sich wieder eine Libellenlarve in meinem Aquarium, doch habe ich bei ihr noch nicht viel Erfolg mit meinen Fütterungsversuchen gehabt."

CONCLUSION

In conclusion it might be said that we have three types of experiments with large numbers of *H. interpunctata* nymphs which clearly show the power of formation of associations by three distinct processes. In case of the investigations on the reactions to objects it was found that the insect gradually increased the distance at which it responded positively to objects in an evenly lighted environment. In another set of experiments it gradually inhibited its usual negative response to light and proportionately reinforced its reaction to an object against the rays of light. It is also important to note the fact that this inhibition in phototactic response was only temporary; it occurred only when the object was present, for whenever the object was removed the nymph would manifest its usual dislike for the bright light. In case o. the experimental work on the manifestations of fear through previous experience we have results which can hardly be interpreted in any other way except as a clear example of the associations of my movements with pain, for why should the disturbed specimens show such obvious signs of agitation when the undisturbed group evinced no indications of fear under otherwise practically identical conditions. Lastly, we have results which seem to indicate quite conclusively that the insects associated my presence with food. These results, again, become more convincing when compared with the unvarying individual records of the group not so trained. The foregoing conclusions are not based on the results tabulated in this paper alone, but on the results of a large number of similar experiments performed with many large sets of specimens and continued for two years during the various seasons.

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