Technical Papers

A Thermophobic Insect[1]

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Recent interest in the physiology of organisms known to be tolerant to relatively low temperatures (*1*) prompts me to report observations made in 1948 concerning the life history of the stonefly, *Nemoura columbiana* Claassen (order Plecoptera).

Stoneflies were among the first adult insects observed to be active in the vicinity of Anchorage, Alaska, during the spring of 1948. Specimens of *Nemoura cintipes* Banks and *N. columbiana* Claassen (identified by W. E. Ricker) were taken as early as April 9. They continued to emerge from the smaller streams as rapidly as the ice covering the streams broke up.

On April 19 a stream on a mountainside 17 miles northeast of Anchorage was examined. It was entirely frozen over for one-half mile below the point where the stonefly observations were made. Higher up, the stream flowed down a precipitous slope through a channel buried deep beneath a series of ice cascades. Along the lower part of the stream, water could frequently be heard flowing beneath the ice, and at such places holes were cut so rocks could be withdrawn and examined for black fly larvae.

A short distance below the ice cascades a hole was cut through 1 ft of snow and 2 in. of ice. This hole was found to open into an ice cavern that had been eroded in the ice immediately over a riffle in the stream. On the ceiling of the ice-enclosed cell were 2 mating pairs and 19 individual specimens of *N. columbiana* Claassen. Three individuals were seen to fly out of the hole and land near by on the snow, where they crawled actively about.

The temperature of the water in the stream and of the air inside the cell was 32° F. The air temperature outside was 30° F. An overcast sky minimized any chance that the dark-bodied insects might be warmed to some extent by solar radiation. These insects appeared to be conducting themselves normally and enjoying full use of their body functions at a temperature of 32° F. The ice covering this part of the stream did not break up until about 10 days later, and had the insects been left undisturbed there is good reason to believe that they could have deposited eggs and died without escaping into the open air.

Weekly temperature records were kept for this stream from April 19 through October 26. The highest temperature recorded was 44° F, and the average of all records was 38.03° F. From these stream temperature records, and the observations concerning the adults, it would appear that these insects have a remarkable capacity for growth and function at temperatures that are continuously near the level at which development of most insects ceases.

In view of the well-known tendency of poikilothermic animals to prolong their period of development when subjected to temperatures below optimum, it might be expected that *N. columbiana* and related species of *Nemoura* would require more than one season to complete their development. Available evidence (*2, 3*), however, indicates that the species of *Nemoura* have one generation a year. If this is true, these insects should prove worth-while subjects for study by workers interested in the physiology of organisms living under conditions of quite low temperature.

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[1] These observations were made incidentally during the course of studies concerned with the biology of Alaskan black flies. These, and associated investigations, were conducted by the Bureau of Entomology and Plant Quarantine, USDA, under a transfer of funds from the National Military Establishment.

The Effect of Reinforcement History on Extinction after Reconditioning

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This paper deals with an experiment to determine whether extinction to operant (preconditioning) level is permanently effective in eliminating differences in response strength among groups given varying amounts of original conditioning. Resistance to extinction after a constant amount of reconditioning was used to test the effectiveness of the preceding "complete" extinction. Nearly all reported studies of reconditioning have clearly involved only partial interpolated extinctions, as in periodic or aperiodic reinforcement (*1–4*). Two reported experiments in which a criterion of complete extinction was met (*5, 6*) were concerned with avoidance conditioning, and are, thus, not directly pertinent to the present study. Nevertheless, in one of these (*6*), the data for

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[1] Postdoctoral fellow, National Research Council, 1950.

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TABLE 1

SUMMARY OF DATA

(The asterisked values in extinction are performances on the first criterion day)

GROUPS	Control (N = 8)			I (N = 5)			II (N = 6)			III (N = 7)			IV (N = 7)		
Number of reinforcements in original conditioning	0			12			30			65			125		
	Mean	*Median*	σ	*Mean*	*Median*	σ	*Mean*	*Median*	σ	*Mean*	*Median*	σ	*Mean*	*Median*	σ
Operant level (based on days 5 and 6)	20.3	20.0	11.1	21.4	20.0	10.9	23.5	21.7	14.3	19.4	18.0	11.3	21.6	14.5	15.4
Daily responses during extinction after original conditioning															
Days															
1	37.0	35.5	13.6	29.8*	26.0*	18.6*	72.8	69.5	15.0	81.0	82.0	17.7	90.7	97.0	33.0
2	34.5	33.0	14.3				45.5	30.5	20.1	47.9	40.0	13.5	73.0	68.0	41.5
3	33.8*	32.0*	23.4*				39.8	32.0	19.3	36.7	34.0	13.8	58.9	62.0	10.8
4							34.7*	31.0*	18.2*	26.3*	24.0*	7.3*	44.6	44.0	12.8
5													51.1	48.0	31.8
6													50.9	50.0	23.5
7													40.4	41.0	18.9
8													43.7	50.0	19.4
9													35.0*	34.0*	20.4*
Daily responses during extinction after reconditioning															
Days															
1	55.3	55.5	20.6	35.8	39.0	15.9	53.7	44.0	24.5	50.1	44.0	18.3	50.4	37.0	42.5
2	33.7	33.0	14.9	23.4	21.0	8.3	50.1	35.5	36.0	34.7	32.0	11.7	39.7	28.0	28.2
Total (Days 1 + 2)	89.0	88.0	33.1	59.2	54.0	22.5	103.8	81.5	50.9	84.8	73.0	23.7	90.1	67.0	70.2

extinctions following successive reconditionings show no tendency for resistance to extinction to increase.

The subjects of the present experiment were 40 male albino rats, Wistar stock, 100–120 days old at the start of experimentation. These were divided into 5 groups of 8 rats each. Subsequent deaths reduced the size of the groups to the *N*'s given in Table 1. The apparatus consisted of living-experimental cages, response levers, and recording devices as described by various authors (*2*, *7*, *8*). The general experimental conditions were those detailed by Notterman (*2*). The entire experiment was performed on consecutive days.

Three days were used to begin the establishment of a hunger rhythm, involving 23¼ hr of deprivation and 45 min of feeding. Starting with the fourth day of rhythm, operant level sessions of 45 min duration were given on each of the next 6 days. They served two main purposes: To ensure that the experimental groups were not significantly different in pretraining bar-pressing activity, and to provide a criterion for “completeness” of later extinction (*9*). For the experimental groups there followed 5 consecutive days of conditioning with regular reinforcement. On day 1, 10 pellets were dropped singly by the experimenter into the food tray to train a tray-approach response at the sound of a falling pellet. The bar was then inserted, and each bar depression was reinforced with a pellet until the animal had received ⅕ the total number of reinforcements allotted members of his group. On each of the 4 subsequent conditioning days, animals were again given ⅕ their allotted total of reinforcements for bar pressing. Groups I, II, III, and IV were assigned totals of 10, 30, 65, and 125 reinforcements, respectively. The training for the control group involved no bar-pressing, but consisted entirely of the delivery of pellets singly and at intervals of about 30 sec to the food tray. Each pellet delivery was, however, preceded by a bar-click, produced by the experimenter. Thirty-five pellets were delivered on day 1, and 25 pellets on each of the next 4 days. The daily schedule of pellet delivery was thus the same as the schedule of bar-pressing reinforcements for Group IV.

After the last conditioning session, daily extinction sessions of 45 min duration per animal were begun. For each group (including the control), extinction lasted until 3 out of 4 successive sessions failed to yield bar-pressing totals significantly greater (at 0.05 level) than that group's operant level. To obtain an operant level criterion, the responses made by each animal on days 5 and 6 of the operant level phase were averaged, and group means were then obtained (*9*). On the day after a group had met its criterion, reconditioning took place. Ten pellets were dropped singly and at intervals, as before, into the food tray to reinstate tray approach, and, after insertion of the bar, pressing responses were regularly reinforced until 15 reinforcements had been given. Extinction was then resumed for 2 days, with one 45-min session per day.

The data are summarized in Table 1. As indicated there, operant level was approximately the same for all groups, in spite of the previously mentioned loss of animals. Means and medians are also presented for daily response totals obtained during extinction after original conditioning. The asterisked values for each group are those for the first criterion day. Thus, only one value is recorded for Group I, since it failed, even on extinction day 1, to give a mean response total significantly higher than its mean operant level. The effect of varying the number of reinforcements during original conditioning is seen, for example, in the extinction day 1 means. The values for Group II, III, and IV are significantly higher (0.01 level) than those for the control group and Group I, though they fail to differ significantly (0.05 level) from one another. There is little doubt that Group IV had acquired considerably greater response strength as a result of original conditioning than had the other groups. For 8 successive days of extinction it gave a mean response total significantly greater (0.05 level) than its mean operant level. This may be compared with 3 days for Groups II and III, 2 days for the control group, and no days for Group I.

That the control group gives values significantly higher than its operant level on extinction days 1 and 2 may be ascribed to (1) an increased tendency to remain in the food tray area where the lever was situated, and (2) the secondary reinforcing power acquired by the bar-click through repeated correlation with the delivery of pellets.

At the bottom of Table 1 are presented the means and medians for each of the 2 extinction days that followed reconditioning, as well as for the 2-day totals. Neither the daily nor the 2-day values yield significant (0.05 level) differences. The lower values for Group I may be related to the relatively low response level it had reached by the last of the criterion sessions (not shown in Table 1).

Thus, significant differences obtained during the first extinction fail to reappear during post-reconditioning extinction. Nor is any trend discernible. Accordingly, the tentative conclusion of the study is that "complete" extinction to operant level tends to eliminate permanently differences in response strength produced by varied histories of reinforcement. That all effects of previous conditioning may not be lost, however, is suggested by the report that successive reconditionings require progressively less time and fewer trials (*5, 6*). The present finding, if valid, permits the use of experimental designs based upon the assumption that extinction to operant level removes the effects of differential intergroup exposure to such independent variables in acquisition as number of reinforcements and, possibly, amount of reinforcement (*7*).

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Microscopic Structure of Carrot Chromoplasts

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Carotene appears inside carrot cells in regular, crystalline bodies, the nature of which has remained uncertain until recently. Following the studies made by P. Fritsch (*1*), A. Meyer (*2*), W. Schimper (*3*), and M. Courchet (*4*) of these red particles inside the cells, the carotene bodies were considered by most botanists to be carotene crystals formed by plastids. Nothing, or at least very little, of the plastid substance was believed to have remained combined with the carotene bodies. This opinion was based not only on the crystalline form of the particles, but also on their cytochemical properties. For, when carrot slices are treated with lipid solvents on a microscopic slide, the red bodies can be seen under the microscope to dissolve, and no residue, or only an insignificant amount, remains. Recently T. E. Weier (*5*) again undertook a detailed cytochemical study of the carotene bodies of carrots. He, too, thought that "the larger bodies are distinctly carotene crystals. There is some question as to whether the smaller ones are. . . ." Weier observed, however, that the pigment may be associated with starch grains and that it is then present in the cytoplasm surrounding the starch and does not always appear crystalline. Weier believed that this pigment-starch-cytoplasm complex was probably a plastid.

In 1939, while studying the physical state of the carotene in carrot juice, the author saw carotene bodies for the first time but did not regard them as carotene crystals (*6*). In 1942 and 1943 he attempted to isolate the red particles from carrot juice, but was only partially successful (*7, 8*). Cytoplasmic granules of microscopic and submicroscopic size remained obstinately adsorbed to the carotene bodies during all steps of purification. Although most of the granules could be removed, a certain number of them still contaminated the final preparations. (Only now can it be recognized that the contamination with submicroscopic, cytoplasmic granules was much higher than had been estimated at that time.) The purest preparations made at that time contained about 5% carotene, 40% proteins, and 45% lipids (*8*).

Recently the author has been able to resume his study of these carotene bodies.[1] A new method has made it possible to separate the chromoplasts from almost all cytoplasmic granules, as may be seen in Fig. 1. The new preparations contain 20–50% carotene, varying with the season and perhaps other factors. Their chemical composition and other properties will be described later.

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