Acquisition and Extinction of a Shuttling Response in Honeybees 
*(Apis mellifera)* As a Function of the Probability of Reward

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The effects of partial (intermittent) versus consistent reward on the acquisition and extinction of a shuttling response were studied in 3 experiments with foraging honeybees (*Apis mellifera*). Adding nonrewarded trials to rewarded trials (the equated-reinforcements design) improved performance in acquisition and increased resistance to extinction. Substituting nonrewarded trials for some rewarded trials (the equated-trials design), which had little effect on acquisition, also increased resistance to extinction but to a lesser extent than adding nonrewarded trials. Marked variations in the schedule of partial reward (the sequence of rewarded and nonrewarded trials) were without effect. The results are compared with those of analogous experiments on vertebrates.

One of the many phenomena of vertebrate learning that have been found in comparative experiments with honeybees (Bitterman, 1988) is the partial reinforcement effect (PRE; or the greater resistance to extinction after training with partial or intermittent reward than after training with continuous or consistent reward). Considerable attention has been paid to the PRE in the vertebrate literature (Mackintosh, 1974) because of its challenge to prevalent conceptions of the role of reward and nonreward in learning and of the nature of extinction. The PRE in honeybees was found first in a free-operant situation designed by Grossmann (1973), who trained individual foragers to dip into a hole for sucrose solution and rewarded the animals of one group for each response and those of a second group for every fifth response. The second group responded more rapidly than the first group both in training and extinction. The PRE was found also by Sigurdson (1981) in a test of the efficacy of a fully automated, discrete-trials training situation for honeybees with latency of dipping as the measure of performance. The partially reinforced animals, which were permitted the same number of responses as the continuously reinforced animals but rewarded for only half of them, responded less rapidly in training than the continuously reinforced animals but showed greater resistance to extinction. Resistance to extinction in rats can be increased simply by delaying reward on half the training trials, rather than by withholding it entirely (Crum, Brown, & Bitterman, 1951), and the same has been found to be true of honeybees (Couvillon & Bitterman, 1980).

A clear implication of the PRE is that the sensory correlates of reward (R) and nonreward (N) acquire discriminative control of performance. In massed training (that is, in training with brief intertrial intervals), control may be exerted by such stimuli as food particles in the mouth and feedback from persisting emotional response or by traces, that is, short-term memories, of those stimuli (continuously decaying afferent residuals) that carry over from trial to trial. One early explanation of the PRE was that partially reinforced animals are rewarded in training for response to the aftereffects of N when R trials follow N trials, aftereffects that are encountered again in extinction (Hull, 1952; Sheffield, 1949). In fishes of two different species, goldfish (Couvillon, 1984; Schutz & Bitterman, 1969) and African mouthbreeders (Longo & Bitterman, 1960), the PRE is abolished when sensory carryover is eliminated by spacing the training trials widely or is disrupted by interpolating R and N trials with other stimuli. In pigeons and rats, however, the PRE survives both manipulations (e.g., Couvillon, Brandon, Woodard, & Bitterman, 1980; Roberts, Bullock, & Bitterman, 1963; Weinstock, 1954), which suggests that something more than sensory carryover is involved. The usual interpretation is that representations of R and N are in one way or another associatively reinstated (Amesel, 1958; Capaldi, 1971).

Long intertrial intervals are not feasible in work with honeybees, but in a series of interpolated-trials experiments designed to test the carryover interpretation, the PRE failed to appear when R trials with the partially rewarded stimulus were never preceded by N trials (Ammon, Abramson, & Bitterman, 1986), another familiar vertebrate outcome (e.g., Spivey & Hess, 1968). In the same series of experiments, the importance of carryover was indicated more directly by the fact that there was more response in extinction to a continuously rewarded stimulus when it was preceded in training by a partially rewarded stimulus than when it was preceded by a continuously rewarded stimulus, a result reminiscent of the generalized PRE in rats (e.g., Brown & Logan, 1965). The present experiments, patterned again after work with vertebrates, were designed to provide some further information about the PRE in honeybees under training conditions thought to be more
appropriate for sustained functional analysis than those used previously.

Experiment 1

The earliest evidence of the discriminative control of performance by R and N in vertebrates was provided by a massed-trials runway experiment with rats in which R and N trials were singly alternated (Tyler, Wortz, & Bitterman, 1953). At the outset the animals ran more rapidly after R than after N, but as training continued, they began to run more rapidly after N than after R, and they showed less resistance to extinction than animals trained with R and N trials in quasi-random sequence. One of our purposes in this experiment was to try to duplicate those results. At the same time it was convenient to study the effect of N-length (the number of successive N trials in training), which significantly affects resistance to extinction in vertebrates (e.g., Gonzalez & Bitterman, 1964; Gonzalez, Eskin, & Bitterman, 1963).

The carryover interpretation of the influence of N-length is that the aftereffects of N cumulate in massed trials and that animals trained with long sequences of N trials are rewarded for responding to those cumulative effects, which they will encounter again in extinction. In all, four groups of honeybees were trained, three partially reinforced groups and a continuously reinforced control group. One of the partially reinforced groups was trained with an alternating schedule, and the other two were trained with quasi-random schedules that differed in N-length.

Method

Subjects. The subjects were 40 foraging honeybees (Apis mellifera), all experimentally naive, from our own hives situated near the laboratory. Trained individually, they were assigned to four experimental groups in quasi-random fashion.

Procedure. The training situation was like that developed by Lee and Bitterman (1990). It consisted of two immediately adjacent windows (each 55 cm wide and 55 cm high) separated by a thin (2 cm) wooden partition around which the animal was required to fly from one window to the other. In the pretraining, experience with both windows was given. From a group of foragers at a feeding station that provided 10%-15% sucrose solution, a single animal selected at random was carried to the laboratory and set down at a large drop (>100 μl) of 50% sucrose solution on a 2.5 x 2.5 cm square target of orange plastic. The target was centered on the shelf of one of the two windows (the left for half of the subjects and the right for the rest). There the animal was marked with a spot of colored lacquer as it fed to repletion, after which it was permitted to leave for the hive. Typically, the animal returned to the laboratory after a few minutes, continuing to forage there as long as sucrose was available. If the marked animal did not return to the window after its first placement, it was carried there from the feeding station, where it usually could be found, and placed again on the target. When the animal did return to the first window, it was picked up after a few seconds of feeding and placed on an identical target centered on the sill of the alternative window, where it was permitted to feed to repletion. On subsequent visits a target was presented twice at the second window and once more at the first. The pretraining ended after the animal had returned twice to each window of its own accord.

The training targets, which were the same as those used in the pretraining, were selected haphazardly from a large set of identical targets to which they were returned after washing at the conclusion of the visit; the purpose of this procedure was to randomize irrelevant odors. Arriving from the hive on each of 12 training visits, an animal found a target centered on the shelf of one of the windows, the left on half the visits and the right on the rest, in quasi-random order. The target contained a 10-μl drop either of 50% sucrose solution (R trial) or of water, which was unacceptable to the animal and distinguishable from the sucrose only by taste (N trial). When the animal landed on the target, a second target that contained a 10-μl drop either of sucrose solution or water was placed on the shelf of the adjoining window, to which the animal flew after leaving the first target. The time (in seconds) between leaving the first target (which the experimenter signalled by pressing a button) and landing on the second target (signalled by pressing the same button), defined as the latency of response, was measured and recorded by a computer. Then the first target was removed, a third target was placed on the shelf of the arrival window, the time between departure from the second target and landing on the third was measured, and so forth, until (after about 5 reward trials on average) the animal was replete and left of its own accord for the hive.

The animals of the continuously reinforced Group C (n = 10) had only R trials, an average of about 60 in all on the 12 training visits. The experimenter could control exactly only the number of visits; the number of trials on each visit was determined by the subject, which was free at any time to return to the hive but which usually averaged about 50 μl of sucrose (5 rewards) per visit. The time between visits averaged about 4 min. The animals of the remaining three groups, in addition to the approximately 60 R trials, about 60 N trials (5 per visit on average). For the partially reinforced Group PA (n = 9), R and N trials were singly alternated, half the visits beginning with R trials and half with N trials in balanced order. The quasi-random reward schedule for the partially reinforced Group PS (n = 10) had almost equal numbers of N–R, R–N, N–N, and R–R transitions, with both N-length and R-length (the number of R trials in succession) varying, respectively, between 1 and 3 with a mean of 1.8. Half of the visits began with an R trial and half with an N trial in balanced order. The schedule for the partially reinforced Group PL (n = 11) was very much like that for Group PS in all respects except for the distribution of N-lengths, which varied between 1 and 7 with a mean of 1.9 and increased gradually as training continued. It may be well to note that in studying nonreward length it is essential to equate for number of N–R transitions, which is in itself a determinant of resistance to extinction in rats (e.g., Spivey & Hess, 1968), although the two variables were of necessity confounded in the comparison of alternating and quasi-random schedules.

On the visit after the 12th training visit, the targets contained only 10-μl drops of water for all groups, and the number of shuttling responses in each of 20 successive 30-s intervals (a 10-min period in all) was recorded. Response was defined as the first contact with a target in one window after contact with a target in the other. With no reinforcement on any trial, the pace of the experiment increased sharply at first, which made the measurement of latency impractical. To measure shuttling it was necessary only to register arrival at each target, but to measure latency it was necessary to register both arrival and departure, which, along with the other things to be done in the 10-min period, made too great a demand on the experimenter.

Results

In acquisition, the shuttling behavior was erratic at first, as indicated by the long initial latencies, but gradually became
more efficient. Group PA showed a very small tendency to respond less rapidly after R than after N, which was not a product of the alternation schedule, because the other two partially reinforced groups did so also. Analysis of variance for the latency data of the three partially reinforced groups yielded a significant carryover effect (after R vs. after N), $F(1, 27) = 14.61, p = .0007$, and a significant effect of visits, $F(11, 297) = 108.45, p < .0001$, but neither a significant group effect, $F(2, 27) = 1.50, p = .2416$, nor a significant Carryover x Group interaction, $F(2, 27) < 1$. The carryover effect is shown in Figure 1, which is plotted in terms of mean natural log (ln) latency of response on trials after R and trials after N for each training visit. The tendency in all three groups to respond less rapidly after R trials, rather small except at the outset of training—$F(11, 22) = 5.10, p < .0001$, for the Carryover x Visit interaction—is reminiscent of the frustration effect in rats, which run less rapidly in the second alley of a double runway after reward as compared with nonreward in the goal box of the first (Amsel & Roussel, 1952). The carryover seems best understood in nonassociative terms as either due to transient competition with shuttling produced by feeding or due to transient facilitation produced by the encounter with water.

Figure 2, which is plotted in terms of mean ln latency per visit, shows that the combined performance of the partially reinforced groups was better than that of the continuously reinforced group. Analysis of variance yielded a significant consistency (partial vs. continuous reinforcement) effect, $F(1, 38) = 19.05, p = .0001$, and a significant visit effect, $F(11, 418) = 100.41, p < .0001$, with no significant interaction, $F(11, 418) = 1.18, p = .2971$. That the performance of the partially reinforced animals was superior simply because they had twice as many training trials per visit as the continuously reinforced animals (on average, 10 rather than 5 trials) is indicated by Figure 3, in which mean ln latency is plotted over the first 6 visits for the partially reinforced groups combined and over 6 blocks of 2 visits for the continuously reinforced group, that is, over blocks that averaged about 10 trials. Analysis of variance yielded a significant block effect, $F(5, 190) = 114.40, p < .0001$, but neither a significant consistency effect, $F(1, 38) < 1$, nor a significant Consistency x Block interaction, $F(5, 190) < 1$. These results suggest that interpolated N trials strengthened the shuttling tendency as much as did R trials.

In Figure 4, the course of extinction in the four groups is plotted (as is the convention in this laboratory) in terms of the mean cumulative number of responses in successive 30-s intervals. Analysis of variance (based on the uncumulated data) yielded a significant group effect, $F(3, 36) = 5.71, p = .0027$, and a significant effect of 2.5-min blocks, $F(3, 108) = 35.32, p < .0001$, with a nonsignificant Group x Block interaction, $F(9, 108) = 1.28, p = .2606$. A separate analysis of the data for the three partially reinforced groups yielded a significant block effect, $F(3, 81) = 23.90, p < .0001$, with neither a significant group effect, $F(2, 27) < 1$, nor a significant Group
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3.5-1
3.0-2.5-
2.0-1.5-
10-TRIAL BLOCKS

Figure 3. Mean natural log latency of response in blocks of approximately 10 trials in Experiment 1 for the continuously reinforced group (C) and the partially reinforced groups combined (P).

× Block interaction, \( F(6, 81) = 1.45, p = .2071 \). A comparison of the continuously reinforced group with the three partially reinforced groups combined yielded a significant consistency effect, \( F(1, 38) = 14.97, p = .0004 \), and a significant effect of 2.5-min blocks, \( F(3, 114) = 25.10, p < .0001 \), with a nonsignificant Consistency × Block interaction, \( F(3, 114) < 1 \). The additional N trials given the partially reinforced animals may be said therefore to have produced a stronger shuttling tendency that was reflected both in more rapid responding at the conclusion of training and in greater resistance to extinction. These results are in accord with the free-operant results of Grossmann (1973), whose partially reinforced animals, given the same number of reinforcements as the continuously reinforced animals, made more responses both in training and in extinction. There were, however, no differential effects of the three partial schedules either on performance in training or on resistance to extinction.

Experiment 2

In comparing partial and continuous reward, either the number of reinforcements (R trials) can be equated, as in Experiment 1 and in Grossmann's (1973) experiment, or the total number of training trials can be equated, as in Sigurdson's (1981) experiment, but not both. The equated-reinforcements PRE may seem to follow naturally from the facilitating effect on performance of the added N trials in training. The equated-trials PRE, to which much more attention has been paid in the vertebrate literature, presents a more difficult problem, especially when the performance of the partially reinforced animals at the conclusion of training is poorer than that of the continuously reinforced animals, as was the case in Sigurdson's experiment. The equated-trials PRE may be thought of as a true PRE in the sense that it seems to be a consequence of inconsistency of reward per se.

Our primary interest in Experiment 1 was in the effects of varying the schedule of partial reinforcement. We used a continuously reinforced group simply to provide a frame of reference, and we chose to equate the number of visits (and therefore the number of reinforcements) rather than to confound number of visits with consistency of reinforcement. Given the lack of a schedule effect in Experiment 1 and the fact that an equated-trials PRE in honeybees had been demonstrated only once before (in the exploratory work by Sigurdson, 1981), it seemed reasonable next to ask whether we could find an equated-trials PRE under our own, much more efficient, experimental conditions. The plan was to use a single partially reinforced group with a schedule like that of Group PS in Experiment 1, together with two continuously reinforced groups, one with the same number of visits (equated reinforcements) and the other with twice the number of visits (equated trials). The duration of the extinction test was increased to 15 min because extinction had not seemed quite complete in the 10-min period of Experiment 1.

Figure 4. Mean cumulative number of responses in extinction for the four groups of Experiment 1. (C = continuously reinforced; PA = partially reinforced, alternating R and N trials; PS = partially reinforced, quasi-random schedule; and PL = partially reinforced, quasi-random schedule with greater N-lengths.)
Method

Subjects. The subjects were 29 foraging honeybees, all experimentally naive, from our own hives. They were trained individually and assigned in quasi-random fashion to three experimental groups.

Procedure. The experimental situation and the pretraining procedure were the same as in Experiment 1. There were two continuously reinforced groups that were trained as in Experiment 1, except that one of them had 9 visits (Group C-9, \( n = 10 \)) and the other had 18 visits (Group C-18, \( n = 9 \)). There was also a partially reinforced group (Group P, \( n = 10 \)) trained with much the same schedule as Group PS of Experiment 1 but in 9 visits. The extinction test for each group, which was given on the visit after each subject's last training visit, was the same as in Experiment 1 but 15 min instead of 10 min in duration.

Results

In Figure 5, the course of learning in Groups P and C-9 is plotted in terms of mean In latency of response on each training visit. Here again, as in Experiment 1, partially reinforced training produced better performance than continuously reinforced training with the number of reinforcements equated. Analysis of variance yielded a significant group effect, \( F(1, 18) = 11.89, p = .0029 \), and a significant visit effect, \( F(8, 144) = 45.79, p < .0001 \), without a significant Group \( \times \) Visit interaction, \( F(8, 44) < 1 \). The partially reinforced animals had about twice the total number of training trials, and Figure 6, which compares the performance of Groups C-9 and C-18, shows clearly how continuously reinforced performance was increased by doubling the number of training trials. In Figure 7, the course of learning in Groups P and C-18, which had the same number of training trials, is plotted in terms of mean In latency of response per visit for Group P and per block of 2 visits for Group C-18, that is, per block of about 10 trials for both groups. The curves suggest that withholding reward on half the trials had little influence on response strength. Analysis of variance yielded a significant block effect, \( F(8, 136) = 50.91, p < .0001 \), with no significant group effect, \( F(1, 17) < 1 \), and only a suggestion of a Group \( \times \) Block interaction, \( F(8, 136) = 1.93, p = .0600 \). As in Experiment 1, the partially reinforced animals showed a small but reliable tendency in training to respond more rapidly after \( N \) trials than after \( R \) trials, \( F(1, 9) = 11.07, p = .0088 \), which again—because of the quasi-random reward schedule—is unlikely to be associative in origin.

In Figure 8, the course of extinction in the three groups is plotted in terms of the mean cumulative number of responses in successive 30-s intervals. Analysis of variance yielded a significant group effect, \( F(2, 26) = 22.17, p < .0001 \), and a significant 2.5-min block effect, \( F(5, 130) = 28.60, p < .0001 \), with no significant Group \( \times \) Block interaction, \( F(10, 130) < 1 \). Comparison of the data for the two continuously reinforced groups yielded a significant increase in resistance to extinction with the number of training trials, \( F(1, 17) = 22.61, p = .0002 \). Comparison of the data for Group P and Group C-9 yielded a significant equated-reinforcements PRE, \( F(1, 18) = 40.71, p < .0001 \). Comparison of the data for Group P
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Figure 7. Mean natural log latency of response in blocks of approximately 10 trials for Groups C-18 (continuously reinforced in 18 visits) and P (partially reinforced) of Experiment 2.

and Group C-18 yielded an equated-trials PRE that was not quite significant at the conventional 5% level, $F(1, 17) = 4.23, p = .055$.

Experiment 3

In this experiment we looked again for an equated-trials PRE. Two groups were trained in the same way as were the partially and continuously reinforced groups of Experiment 2, but the number of training visits was increased somewhat in an effort to sharpen the effect.

Method

Subjects. The subjects were 34 foraging honeybees, all experimentally naive, from our own hives. They were trained individually and assigned quasi-randomly to two groups.

Procedure. The experimental situation and all the general features of the training and extinction procedures were the same as in Experiment 2. The partially reinforced group (Group P, $n = 18$) was trained as was the partially reinforced group of Experiment 2 but in 12 visits. The continuously reinforced group (Group C, $n = 16$) was trained as were the continuously reinforced groups of Experiment 2 but in 24 visits. Each animal had a 15-min extinction test on the visit after its last training visit. The only unusual feature of the experiment was that it was done in two balanced replications by two different experimenters. One experimenter trained about half the animals in each group, and a second experimenter trained the rest about 3 months later. The total number of animals had to be larger than planned originally in order to give equal weight to the contributions of the two experimenters.

Results

In Figure 9, the course of learning in the two groups is plotted in terms of mean ln latency of response per visit for Group P and per block of 2 visits for Group C, that is, per block of about 10 trials for both groups. These curves do begin to show some deleterious effect on performance of withholding reward on half the training trials. Analysis of variance yielded a significant block effect, $F(11, 352) = 98.59, p < .0001$, with no significant group effect, $F(1, 32) = 2.04, p = .1634$, but a significant Group x Block interaction, $F(11, 352) = 1.87, p = .0428$. As in the previous experiments, the partially reinforced animals showed a small but reliable tendency in training to respond more rapidly after N trials than after R trials, $F(1, 17) = 6.52, p = .0206$.

In Figure 10, the course of extinction in the two groups is plotted in terms of the mean cumulative number of responses in successive 30-s intervals. Analysis of variance yielded a significant group effect, $F(1, 30) = 4.38, p = .0448$, that is, more responding by the partially reinforced animals despite poorer performance at the end of training, and a significant 2.5-min block effect, $F(5, 150) = 37.67, p < .0001$, without a significant Group x Block interaction, $F(5, 150) = 1.41, p = .2248$. The effect of the experimenters was not significant, $F(1, 30) < 1$, nor was the Group x Experimenter interaction, $F(1, 30) = 1.28, p = .2667$. 

Figure 8. Mean cumulative number of responses in extinction for the three groups of Experiment 2. (C-9 = continuously reinforced in 9 visits; C-18 = continuously reinforced in 18 visits; and P = partially reinforced.)
Discussion

Experiments 1 and 2 provide striking demonstrations of the equated-reinforcements PRE in honeybees. In each case, adding N trials to R trials improved both performance in acquisition and resistance to extinction. The phenomenon is, of course, well known in vertebrates, primarily from free-operant experiments (in which reinforcements typically are equated) but also from discrete-trials experiments (in which trials usually are equated). In Notterman's (1951) work with rats in a runway, for example, asymptotic running speed in acquisition increased with the number of added N trials, and so also did resistance to extinction. The improvement of performance in acquisition by added N trials has been explained in terms of secondary reinforcement (Denny, 1946) and on the assumption that associative strength increases with frequency of response per se (Humphreys, 1943). It has been suggested, too, that under certain circumstances the improvement may be artifactual, a byproduct of the redefinition of response by the partial schedule (Mowrer & Jones, 1945). Performance is not always improved by added N trials, and in recent work with octopuses, drastic impairment actually was found instead (Papini & Bitterman, 1991). Nevertheless, when added N trials do improve performance in acquisition, increased resistance to extinction may seem to present no further explanatory problem.

To account for the equated-trials PRE in Experiments 2 and 3, it is necessary, however, to look beyond terminal performance in acquisition, which was equal in Groups P and C-18 of Experiment 2 and was better in Group C than in Group P of Experiment 3. Unfortunately, the schedule variations in Experiment 1, which were suggested by the vertebrate literature and designed to test the hypothesis that the same principles apply to honeybees, yielded only null results, although the null results cannot, of course, be taken to mean that the principles are different. Single-alternation patterning has both appeared and failed to appear in work with pigeons (Couvillon et al., 1980; Roberts et al., 1963) and fishes (Couvillon & Bitterman, 1981; Gonzalez, Eskin, & Bitterman, 1961). Inconsistent results have been obtained in work with rats, and such findings begin to delineate the conditions necessary for patterning. Amount of training is critical (Capaldi, 1958), as are magnitude of reward and effortfulness of response (Gonzalez, Bainbridge, & Bitterman, 1966), and resistance to extinction has even been found to depend on whether the training begins with an R or an N trial (Ishida, 1986). As to the N-length effect, which must be no less parameter-dependent than the alternation effect, there has not been enough systematic study of vertebrates to guide the work with honeybees. Whatever the determinants of the equated-trials PRE may prove to be, it is likely that they contribute as well to the much greater equated-reinforcements PRE.

Given the small amount of work that has been done on partial reinforcement in honeybees (minuscule by comparison with the amount of work on partial reinforcement in vertebrates), the limited present understanding of it is not surprising. In any case, the empirical rules established thus far—that
addition N trials may improve rather than impair instrumental performance and that they may produce greater resistance to extinction than an equal number of R trials, which may have improved performance even more—do provide clear direction to further work. The rules are interesting, too, because they are the same for honeybees as for vertebrates, adding to the picture of broad functional commonality that is emerging in the face of what must be considered to be the perfectly reasonable expectation of substantial evolutionary divergence (Bitterman, 1988; Bitterman & Couvillon, 1991).

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