Effects of Pairing of Chicks During the Imprinting Period on Filial Responses to an Imprinting Stimulus

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Most of the previous investigations on imprinting have evaluated the response to the imprinting stimulus in birds who were housed and examined in isolation. It has been generally assumed in these studies that when neonates are given social experience, they imprint on one another, and consequently, are less likely to respond to the imprinting object.

In order to evaluate this assumption, numerous investigations have examined the effects of social experience with siblings on imprinting. It is difficult to compare the results of these studies, since they differ in species of subjects, method, or the response indice which they employed. There are, however, two types of conclusions. One of them is that social experience with siblings interferes with imprinted attachment to a maternal surrogate (4, 5, 6, 7, 8, 12, 15, 24, 28, 29). In contrast, the other is that social experience facilitates imprinting (1, 2, 20, 21, 25).

Furthermore, some studies report that imprinting was inhibited by social experience when subjects remained to be with siblings but was facilitated when subjects were briefly isolated after social experience (3, 7, 11, 27). Moreover, there are some studies which report the interaction between the developmental process of subjects and the effects of social experience on imprinting (13, 14, 16). Thus we have no consistent conclusion about the effects of social experience with siblings on imprinting.

Therefore, we have no clear reason to say that we must include the training and testing of the subject in isolation. Nevertheless, most studies of imprinting have employed the training and testing in which subjects were in isolation. In particular, when we consider the natural settings in which imprinting occurs, this method in isolation is divergent from the natural setting.
In the natural setting, a bird is simultaneously exposed to both parent and siblings, and filial imprinting occurs to both of them. Therefore, the experimental situation in which a subject is simultaneously exposed to both siblings and an imprinting object must also be included in the laboratory study. There are, in fact, some studies which employed this simultaneous exposure in the laboratory (8, 17, 23, 25, 27, 29). However, among these studies, there is no clear conclusion about the effects of social experience on imprinting. Thus, it is necessary to examine the effects in the situation in which subjects are simultaneously exposed to both siblings and a parent object.

Furthermore, in previous studies, interest has focussed on the inhibitory or facilitative effects of social experience on imprinting. Therefore, it is also necessary to investigate some interrelation between birds' responses to siblings and those to an imprinting object.

In the present research, each subject was simultaneously exposed to both one sibling and an imprinting object during the imprinting training. One sibling was used in this study so that we can easily observe the behaviors of each bird. The purpose was to determine the effects of social contact with one sibling during the imprinting period on imprinting to the object. The second aim was to examine the interrelation between each subject's responses to its partner and those to the imprinting stimulus.

**Method**

**Subjects**: Subjects were 34 White Leghorn domestic chicks. Eggs were hatched in isolation in an incubator; each egg was hatched in a separate cardboard compartment so that the newly hatched chicks were visually isolated from one another, though they could not be prevented from hearing the calls emitted by other chicks.

Immediately after hatching, the chicks were transferred from the incubator to the cages. Except during experimental sessions, the chicks were housed in individual rearing cages. In these cages, the chicks could hear, but not see each other.

Owing to the supply provided by the internal yolk sac, chicks do not need food
Imprinting of Chicks

or water for the first two days after hatching, so that it was not necessary to provide either for them. After this time, food and water were provided.

Apparatus: The imprinting apparatus consisted of a black, plywood runway (22 x 210 x 40 cm). The imprinting stimulus consisted of a red cylinder (6 x 5 x 9 cm) and metronome tones pulsing at the rate of 3 pulses/s. This stimulus ran the length of the runway. The tones were emitted via the 8-ohm speaker from the tape recorder. This speaker was attached to the bottom of the red cylinder.

When each imprinting session started, three lights (each 30 watt, white fluorescent bulbs) mounted on the runway were put on, and at the same time, the imprinting stimulus emitting the tones moved. At the end of the session, the lights and the stimulus were put out.

Finally, the Apple IIe microcomputer system was employed to program stimulus presentation and withdrawal and to record observed events.

Procedure: The subjects received four imprinting sessions for the first three days after hatching (except Group A); one session a day was run for 2 days after hatching and the other two sessions were run on Day 3. The first two sessions were run for 15 minutes and the other sessions were run for 10 minutes. However, the chicks in Group A received three sessions over the first three days. In this group, on Day 3, only the third session was run.

The first session was given approximately 8 + 2 hours after hatching. The interval between the sessions, except for that between the third session and the fourth session, was approximately 24 hours. The fourth session was run immediately after the third session. During each session, the imprinting stimulus was continuously exposed to one subject or two subjects. The experimenter observed and recorded the responses the subjects showed during each session.

The chicks were divided into four groups. The different groups received different experience as follows:

1. Group A (N = 7). These birds were run individually during all the sessions.
2. Group B (N = 15). These birds were run individually as the birds in Group A except the third session. In the third session, a pair of chicks were chosen randomly from this group, marked for identification, and then placed on the runway. It was thus only in this session that each chick was simultaneously exposed to both the partner and the imprinting object.
3. Group C (N = 6). In this group, only the third session consisted of observing and recording the response of each subject in individual. In the remaining sessions, each chick was run with a chick from the same group in the same way as the paired session of Group B.

4. Group D (N = 6). Each bird was run with a chick from the same group except the fourth session. In the fourth session, the subjects were run as individuals. The paired sessions of this group were run in the same way as the paired sessions of the two groups above (Group B and Group C).

These four groups were summarized in Fig. 1.

In the imprinting sessions in isolation, the experimenter observed and recorded the amount of time each chick spent following the imprinting stimulus. In the imprinting sessions in pair, two observers observed and recorded the responses of each two chicks, respectively. In the former sessions, the only amount of time of following response was measured as a response index of imprinting. On the other hand, in the latter sessions, not only the duration of following the imprinting stimulus but the frequency of the events as shown below was measured for each bird:

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**Fig. 1.** Experimental design for each group over three days after hatching.
1. Frequency of following the imprinting stimulus (FFS).
2. Frequency of pecking at the partner (FPP).
3. Number of being pecked by the partner (NBPP).
4. Frequency of following or approaching the partner (FFAP).
5. Number of being followed or approached by the partner (NBFP).

In each pair of each group, each subject's partner was not changed over the sessions.

Two types of indices were used for data analysis. One is the value (Following Time Ratio: FTR) represented in percentage of following response. This value is obtained by dividing the total amount of time each chick spent following the stimulus during each session by the interval of each imprinting session and further being multiplied by 100. This index provided a measure of performance for each subject in any one session. A FTR less than 10 (%) indicated less performance. A FTR higher than 10 (%) indicated good performance. The other index is the frequency of each event shown above.

**Results**

Fig. 2 shows the mean FTR for each group.

A one-way ANOVA (repeated measures) was used to determine if the differences in performance among sessions within each group were reliable.

Group A shows the increasing performance over three sessions. However, only the difference between the performance of the first session and that of the third session was significant ($F=14.082; df=1/6; p<0.01$).

Group B shows that the mean FTR in the second session was greater than that in the first session. Further, it shows that the subjects were less responsive in the paired session (the third session) than in the isolated sessions (in particular, the fourth session). There was a significant difference between the performance of two sessions in each case as follows; the first session vs. the second session ($F=14.095; df=1/14; p<0.01$), the first session vs. the third session ($F=8.548; df=1/14; p<0.01$), the first session vs. the fourth session ($F=31.486; df=1/14; p<0.01$), and the third session vs. the fourth session ($F=5.913; df=1/14; p<0.01$).

The second performance of Group C was good in comparison with the
Fig. 2. Mean FTR (following time ratio) for each group over four imprinting sessions (however, three sessions for Group A). The abscissa shows the imprinting sessions. The ordinate shows the mean FTR performance (%).
performance in the first session. Furthermore, also in Group C, the performance under the isolated condition (the third session) was much better than that under the other three paired conditions. There was a statistically significant difference between the performance of two sessions in each case as follows; the first session vs. the second session (F=10.484; df=1/5; p <0.05), the first session vs. the third session (F=12.024; df=1/5; p <0.05), the first session vs. the fourth session (F=9.201; df=1/5; p <0.05), the second session vs. the third session (F=9.421; df=1/5; p <0.05), and the third session vs. the fourth session (F=6.739; df=1/5; p <0.05).

The performance of Group D was also similar to that of Group C. In Group D, the isolated session (the fourth session) was run after the first three paired sessions. The temporal order between the isolated and the paired session on Day 3 was opposite to that of Group C. Nevertheless, it is shown that the performance of Group D is similar to that of Group C. Group D also represents rather less performance in the paired sessions than in the fourth session (isolated session). Further, it appears that the performance in the second session is good in comparison with that in the first session. However, only two cases as mentioned below show the statistically significant differences between the performance of two sessions; the first session vs. the third session (F=20.814; df=1/5; p <0.01) and the first session vs. the fourth session (F=6.948; df=1/5; p <0.01).

Fig.3 represents the comparison between the mean performance of both Group A and Group B under the isolated condition and that of both Group C and Group D under the paired condition over two sessions after hatching. A one-way ANOVA (repeated measures) revealed a significant sessions effect (F=13.281; df=1/32; p <0.01) and a significant condition effect (F=4.806; df=1/32; p <0.01), reflecting the fact that the isolated subjects were more responsive than were the paired subjects. Further, the ANOVA revealed a statistically reliable sessions × condition interaction (F=5.514; df=1/32; p <0.05).

Next, we compared the performance between each group in the third session. This result is shown in Fig.4. In this session, the groups under the isolated condition were Group A and Group C. On the other hand, the groups under the paired condition were Group B and Group D. A one-way ANOVA (non-repeated measures) reveals a significant condition effect (F=5.639; df=1/32; p <0.05),
reflecting the fact that the performance of the groups under the isolated condition is better than that of the groups under the paired condition.

Furthermore, in this session, the comparison between the performance of each group was conducted. A similar one-way ANOVA reveals that there was a reliable difference between the performance of two groups in each case as follows: Group A vs. Group D \( (F=8.468; \text{df}=1/11; p<0.05) \) and Group C vs. Group D \( (F=7.089; \text{df}=1/10; p<0.05) \).

Next, we compared the performance of all groups in the isolated session. On
Imprinting of Chicks

the basis of results in Fig. 2, the comparison was conducted between the performance of the isolated session of each group on Day 3. That is, for Group A and Group C, the performance of the third session and for Group B and Group D, the performance of the fourth session were compared. A two-way ANOVA revealed that the difference between the performance of each group in the isolated session was not significant (F = 1.225; df = 1/30; p > 0.05).

Fig. 4. Mean performance for each group on the third session. In this session both Group A and Group C were run under the isolated condition. In contrast both Group B and Group D were run under the paired condition.
Further, we compared the performance of three groups (Group B, Group C, and Group D) in the paired session on Day 3. From Fig. 2, it seems that the performance of Group B in the third session was better than either that of Group C in the fourth session or that of Group D in the third session. However, there was no significant difference between the performance of each group ($F=1.373$ ; $df=2/24$ ; $p>0.05$). In spite of different experience over the first two sessions, these three groups show the equivalent performance under the paired condition on Day 3.

Now, the KENDALL's rank correlation coefficient (tau) was calculated to investigate the interrelation between the frequency of each event which was measured for each bird in Group B, Group C, and Group D. Seven events were investigated in all. Five events of them were described in Procedure. The other two events are the performance (FTR) under the paired condition on Day 3 and that under the isolated condition on the same day.

As shown in Table 1, the FTR performance under the isolated condition (FTI) correlated highly with both the FTR performance under the paired condition (FTP) and the frequency of following the stimulus (FFS). Further, the FTR under

|     | FTP | FFS  | FPP  | NBPP | FFAP  | NBFP  |
|-----|-----|------|------|------|-------|-------|
| FTI | .447** | .257 | .165 | -.155 | -.029 | -.008 |
| FTP | .591** | .201 | -.180 | .177 | .387** |
| FFS | .004 | -.032 | .339** | .552** |
| FPP | -.639** | .056 | -.040 |
| NBPP| -.040 | .056 |
| FFAP|       |       | .365** |

Note : **: $p<0.01$ (two-tailed)
FTI : FTR under the isolated condition
FTP : FTR under the paired condition
FFS : Frequency of following the imprinting stimulus
FPP : Frequency of pecking at the partner
NBPP : Number of being pecked by the partner
FFAP : Frequency of following or approaching the partner
NBFP : Number of being followed or approached by the partner
the paired condition (FTP) correlated with both the frequency of following the stimulus (FFS) and the number of being followed or approached by the partner (NBFP). The frequency of following the stimulus (FFS) also correlated with both the frequency of following or approaching the partner (FFAP) and the number of being followed or approached by the partner (NBFP). The frequency of following or approaching the partner (FFAP) correlated highly with the number of being followed or approached by the partner (NBFP). On the other hand, the frequency of pecking at the partner (FPP) negatively correlated with the number of being pecked by the partner (NBPP). It means that the bird who frequently pecked at its partner was rarely pecked by its partner. Furthermore, the observation indicated the stable relationship of pecking among two birds in each pair over the pair sessions. All correlations above are significant (p < 0.01, this \( p \) is the estimated two-tailed normal probability corresponding to the approximated normal deviate) except for the correlation between FTI and FFS (p = 0.06).

Next, when we measured the events of both FFAP and NBFP, the situation in which a bird following the stimulus was followed by its partner was frequently observed than that in which a bird not following the stimulus was followed or approached by its partner. A one-way ANOVA (repeated measures) revealed a significant difference between the frequency of occurrence of the both situations \( (F = 7.543; df = 1/27; p < 0.01) \). However, the correlation between the frequency of both situations was not significant (KENDALL's \( \tau = 0.0417; p = 0.78 \)).

Discussion

As shown in Fig. 2, the performance for all groups improved over the first two sessions. It may be that these improvements were due to some training effects or the development of chicks' motor activity. However, the improvement of the performance of both Group A and Group B was greater than that of both Group C and Group D. That is, over the first two sessions, the two groups which were exposed to the stimulus under the isolated condition improved in their performance more than the other two groups which were exposed to the stimulus under the paired condition. What caused this difference of improvement? Since the rearing conditions except for the experimental conditions were identical for
the above four groups, the difference might not be due to either training effects, or to that of the development of the chick's motor activity. Rather, we can consider one major difference in the experimental conditions.

Therefore, over first two sessions, when there is one sibling in the experimental situation where the subject may have an opportunity to imprint to the surrogate object, the presence of this sibling may interfere with the imprinting to the stimulus for the subject.

Next, also in the third session, the performance of the isolated birds was better than that of the paired subjects (see Fig. 4). Further it is of interest that the subjects in Group C which had been under the paired condition during the first two sessions improved their performance under the isolated condition of the third session (see Fig. 2, 4). However, when the experimental condition was returned to the paired condition on the fourth session again, their performance returned to the first level (Fig. 2). This tendency was also found in Group D (Fig. 2). In spite of the low performance under the paired condition, this group also showed good performance under the isolated condition of the fourth session. Moreover, the subjects in Group B followed the imprinting stimulus well under the isolated condition on the fourth session after the paired session (the third session). Therefore, also on Day 3, the presence of one sibling impaired the imprinting to the stimulus for the subject.

From these results, the impairment of the performance under the paired condition for these groups was doubtlessly due to the presence of one sibling in the imprinting situation.

Therefore, the results of the present study support those reported by other investigators (8, 17, 27, 29) who used the simultaneous exposure situation in the same way as this study.

However, why does the presence of one sibling impair the tendency of following the stimulus? It is necessary to elucidate the experimental situation more carefully in order to find out the reason. In the situation where there is one sibling in the imprinting setting, the object toward which the subject responds will not only be the stimulus but also the sibling. Therefore, the more the subject responds to the sibling, the less it responds to the stimulus. If so, it is natural that the presence of one sibling impairs the imprinting. In fact, KLOPFER (15) suggests
that rearing isolates enhances following and that this enhancement would occur by reducing the variety in sensory input. That is, according to him, it would appear that the increasing of the variety in sensory input leads to an impairment of the response to the input that remains. Thus, it would seem that the strength of the imprinting is relative in that its level is partially determined by the stimulus conditions that prevail at a given time, as suggested by HOFFMAN (10).

A more interesting fact is that the subjects in both Group C and Group D showed excellent performance in isolation on Day 3 in spite of low responsiveness to the stimulus over the first two sessions. We consider that they were covertly imprinted to the stimulus. They had poor opportunity to follow the stimulus during the first two days. Nevertheless, it seemed that they had been already fully imprinted to the stimulus at that time. This consideration is consistent with that of GAIONI, DePAULO and HOFFMAN (4). They report that group-reared birds confronted with an imprinting stimulus may become attached to that stimulus, but that evidence of this may be masked by competing response tendencies directed toward siblings and that when the siblings are subsequently removed, the imprinting effect is revealed. Thus, also in the present study, we consider that even under paired conditions, each bird might be imprinted to the stimulus, but that it might be masked by competing responses directed toward a sibling.

When we consider this possibility, we have raised doubts regarding the reliability of following as an index of imprinting strength. HESS (9), POLT and HESS (20), and GAIONI, DePAULO, and HOFFMAN (4) suggest the disadvantage of following as an index of attachment. PETERSON (19) also suggested that the following response is not necessary for presentation of the imprinted stimulus to function as a reinforcer.

The basic mechanism for establishing imprinting appears to be a susceptibility to reinforcement by proximity to the imprinting object, not the formation of following response, as suggested by SKINNER (26). From this point of view, in the present study in spite of poor experience in following the stimulus, the subjects in both Group C and Group D may have already been reinforced by the stimulus during the first two days. This reinforcement may have caused the chicks to follow the stimulus well in the absence of one sibling.

From Table 1, the subjects’ performance (FTI) which was good under the
isolated condition on Day 3 showed a good performance (FTP) also under the paired condition on the same day. Furthermore, they were frequently followed by their partner under the paired condition. Moreover, such subjects frequently followed not only the stimulus but their partners as well. The interrelation between such events was summarized as follows; Stimulus $\leftarrow \text{Sub } X \leftarrow \text{Sub } Y$ (in this diagram the arrow indicates the following response and Sub X is a better follower of the stimulus than Sub Y).

Next, although not significant, the correlation between FTP and FPP seems to be rather large. It shows that the subjects who showed good performance under the paired condition frequently pecked at their partners. Further the subjects which frequently pecked at their partners in the paired session were rarely pecked by their partners. Therefore, we can modify the above diagram as follows; Stimulus $\leftarrow \text{Sub X} \leftarrow \text{Sub Y}$ (in this diagram the mark of $+++$ means the following and/or pecking responses). Similar diagram is reported also by MORIYAMA (18).

Thus far we have considered that Sub Y follows Sub X in the above diagram. In their studies, SMITH and BIRD (29) also suggest the possibility that some of the chicks in the groups might not be responding to the imprinting stimulus but may be following the other chicks of the group who were approaching it.

However, we can also consider the possibility that in the above diagram Sub Y follows not only Sub X but also the stimulus. Furthermore, we can consider the possibility that Sub Y doesn’t follow Sub X but the stimulus. These possibilities are probable from the observation of the events of both FFAP and NBFP. As mentioned in Results, when we measured these events, the situation in which a bird following the stimulus was followed by its partner was observed much more than that in which a bird not following the stimulus was followed or approached by its partner. Moreover, the correlation between the frequency of both the situations was not significant. If Sub Y has a tendency to follow or approach Sub X, we can expect that both situations might have been frequently observed and the correlation between the frequency of them might have been higher.

Thus, when we investigate the situation in which Sub Y runs behind Sub X towards the stimulus in the paired sessions, we must consider the possibility that Sub Y doesn’t run after Sub X, but instead runs after the stimulus.
Imprinting of Chicks

On the other hand, we can consider the possibility of social facilitation. RAMSAY and HESS (22) and COLLIAS and COLLIAS (2) report that naive ducklings were partially imprinted by being placed with well-imprinted ducklings and the imprinting object during the imprinting period. From this point of view, it is considered probable that in the present study not-well-imprinted Sub Y's responsiveness to the stimulus was increased by being paired with well-imprinted Sub X and was elicited by Sub X's responses to the stimulus.

However, we cannot decide which possibility is more probable in this study. More detailed studies of this problem will be necessary.

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Effects of Pairing of Chicks During the Imprinting Period on Filial Responses to an Imprinting Stimulus

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There are several studies which have evaluated the effects of social contact with siblings on imprinting to a maternal surrogate. However, we have no consistent effect of the social experience. Some studies have reported that social experience with siblings interferes with the establishment of imprinting, whereas other studies have reported that social experience with siblings enhances the imprinting experience.

The present study examined the effects of social contact with one sibling during the imprinting sessions on imprinting to the maternal object for chicks (White Leghorn). This experimental situation is similar to the natural setting because under natural conditions imprinting occurs in a social situation which includes a parent and siblings. Next, this study investigated the interrelation between each chick's responses to its partner and those to the imprinting object.

During the imprinting sessions, each chick was individually exposed to only the imprinting object under the isolated condition or exposed to both the stimulus and one sibling under the paired condition over three days after hatching. Each chick showed the better performance under the isolated condition than under the paired condition. Thus the presence of one sibling during the imprinting period weakens the strength of the imprinting.

The more interesting result was that chicks who had showed a poor performance under the first paired sessions followed the stimulus well under the later isolated condition. It seems that these subjects have already fully imprinted to the stimulus in spite of poor experience of following the stimulus. This result renders dubious the significance of following as an index of the strength of imprinting.

Next, it was often observed under paired conditions that as soon as a well-imprinted chick followed the stimulus, the other chick ran behind him quickly. It was also observed that this well-imprinted chick followed its partner and pecked at him frequently. We can consider there is some interrelation between social responses to a sibling and those to a surrogate mother.