Discriminations of Color and Pattern on Artificial Flowers by Male and Female Bumble Bees, *Bombus Impatiens* (Hymenoptera: Apidae)

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ABSTRACT

This study examined the performance of male bumble bees (Bombus impatiens), a species of bumble bee in color and pattern discriminations and compared it to that of female bees. Bees were trained to forage from rewarding (S+) and unrewarding (S-) artificial flowers which differed in color (blue vs yellow) or pattern (e.g., concentric vs radial). Learning of the discrimination by the bees was then assessed by examining choice proportions of different flower types while none of the flowers offered reward. Color discriminations were made with 98% accuracy by the males, and the choice proportion was no different for females. Pattern discriminations were very poor or nonexistent for males but significantly better for females, especially in one of three pattern discriminations (radial vs concentric patterns).

In colonies of honey bees and bumble bees males and females do not share equally in the labor. It is the females, the "workers," that gather nectar and pollen from flowers, bring it back to the colony, and feed it to the larvae. The males perform none of these duties. Once mature, they leave the colony, seek out conspecific queens, and mate. In the species used in the present study, Bombus impatiens Cresson, mating occurs away from colonies, though in other species males may fly to other colonies and mate near the entrance. During that time, they need only collect enough nectar to satisfy their own energetic needs (for a general review of the workings within a colony, see Alford 1975, Free and Butler 1959). Not surprisingly, the study of foraging behavior in social bees has been the study of female bees. Male bees have been studied, but for other reasons. Research topics on males have included mating behavior (e.g., Baer and Schmid-Hempel 1999, Bergman and Bergstrom 1997, Duchateau and Velthuis 1988, Free 1971), territoriality and competition (Alcock and Alcock 1983, O'Neill et al. 1991, Van der Blom 1986), analysis of paternity (Collins and Donoghue 1999, Haberl and Tautz 1998), sex ratios in colonies (Beekman and Van Stratum 1998), flight paths (Jennersten et al. 1991), energy expenditure (Bertsch 1984), spectral sensitivities (Menzel et al. 1988) and neurophysiology (Fahrbach et al. 1997).

A natural extension of this project was to compare the performance of male bees to that of females under comparable conditions. A priori, given that males are not the providers in the colony, there should be a lesser incentive for them to collect food, and we speculated that they might be less adept at learning floral discriminations.

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One impediment to studying male foraging behavior is that they rarely if ever return to the colony once they have left. In a laboratory flight cage, however, they station themselves on the walls and occasionally “swoop down” onto artificial flowers and collect sugar solution. In the following study rewarding (S+) and unrewarding (S-) artificial flowers, differing either in color or in pattern, were arranged in the flight cage. The colors and patterns were chosen on the basis of previous literature: blue vs yellow is commonly used in studies of floral discriminations (e.g. Free 1970, Dukas and Real 1993). Female honey bees also show preferences for radial patterns over concentric patterns and they choose low spatial frequencies over high frequency patterns (Lehrer et al. 1995). Following training, the bees were tested on empty flowers and their choices examined. To ensure that choice of one stimulus over the other could be traced to learning of the discrimination during training rather than some unlearned preference, the S+ and S- were interchanged. The first few choices were examined and the choice proportions for the S+ by the males were compared both to chance levels and to the choice proportions recorded for females.

**MATERIALS AND METHODS**

**Subjects.** Two colonies of bumble bees, *Bombus impatiens* (Cresson), were obtained from Koppert Biological Systems, Inc. (Ann Arbor, Michigan). One colony was used for testing males and the other colony was used later for testing females. Male and female bees were labelled using numbered plastic discs glued to their thorax.

**Flight Cage and Housing.** The bees were trained and tested in a wooden framed, screened flight cage (183 by 188 by 190.5 cm). Males were removed from the first colony, labelled and placed in a small wooden box (20 by 10 by 15 cm) covered with a removable glass plate. A small hole allowed the bees access to the flight cage.

The colony used for testing workers was housed in a wooden container (30 cm by 15 cm by 15 cm) which was connected to a wooden corridor (30 cm long) covered with removable glass plates. The corridor was connected to the flight cage. A vertical plastic gate allowed the experimenter to control which bees entered and exited the colony. Inside the flight cage, radiating black stripes marked the entrance to the corridor to aid workers returning to the colony.

The average temperature in the room was 20°C (range: 18°C-22°C). Illumination on a 12 L: 12 D light: dark cycle was provided by fluorescent lights on the ceiling of the testing room and above the ceiling of the flight cage. We have obtained over 80% success in some subtle pattern discriminations using the same species, the same flight cage, and the same fluorescent lights (Korneluk and Plowright 1995, Plowright 1997, Plowright et al. 2001), notwithstanding the possibility that the flicker in the fluorescent lights is perceived by the insects.

**Artificial Flowers.** Each flower consisted of a wooden rectangular box (12.5 cm long by 8 cm large) supported by a rod (60 cm high). A disc made of construction paper (15 cm diameter) covered in clear plastic vinyl was placed on top of each box. For the first set of visual stimuli, “Yellow/Blue,” three discs were blue and three discs were yellow (Figure 1). In the three following sets, each flower contained both blue and yellow: (1) “Quarters/Sixths,” where all flowers had a radial pattern of alternating blue and yellow, but three of the flowers had alternating sixths and the other three had alternating quarters (Figure 2); (2) “Sixths/Concentric,” where three of the flowers had alternating sixths and three had four alternating blue and yellow concentric circles (Figure 3); (3) “Quarters/Concentric,” where three of the flowers had the “Quarters” pattern and three had the “Concentric” pattern (Figure 4). For each colony, each condition (i.e. each set of visual stimuli) was run once with one pattern as the S+ and the other as the S-, and then once again with the S+ and S- reversed.
Figure 1. Pie graphs displaying the proportions of S+ choices and S- choices for the Blue vs Yellow condition. The total number of observations for each is also given.

![Pie graphs showing S+ and S- choices for the Blue vs Yellow condition.](image)

Legend:  
- Blue
- Yellow

Figure 2. Pie graphs displaying the proportions of S+ choices and S- choices for the Sixths vs Quarters condition. The total number of observations for each is also given.

![Pie graphs showing S+ and S- choices for the Sixths vs Quarters condition.](image)
Figure 3. Pie graphs displaying the proportions of S+ choices and S- choices for the Concentric vs Sixths condition. The total number of observations for each is also given.

Figure 4. Pie graphs displaying the proportions of S+ choices and S- choices for the Concentric vs Quarters condition. The total number of observations for each is also given.
with different bees (so 4 sets of visual stimuli X 2 S+/S- assignments X 2 sexes = 16 combinations).

In the center of each disc was a small plastic vial cap (2.5 cm diameter, approximately 5 ml). During training, caps on rewarding flowers (S+) contained honey solution (2 parts honey to 1 part water by volume), and caps on unrewarding flowers (S-) contained water colored yellow with food coloring. During testing, both types of flowers were unrewarding and contained only colored water. New discs and caps were used after training for the testing conditions.

**Training (Males)**. Bees were given unrestricted access to the flight cage. Upon entering the flight cage, bees would usually fly for a short period of time, land on the sides or ceiling of the flight cage, and feed from the artificial flowers several times per day. Training for each pattern condition lasted for two days, during which time the experimenter would replenish the honey solution in the S+ flowers when these became empty, rinse and wipe clean each cap, and put new water in the S- flowers. The positions of the S+ and S- flowers were changed at the beginning of each day and each time the flowers were refilled. A bee was used in testing only if it had been observed foraging from at least one S+ flower at least once during training. The honey solution was nearly depleted each time the positions of the S+ flowers were changed, indicating that the bees foraged from the S+ flowers.

**Testing (Males)**. After two days of training and each bee was observed foraging at least once, testing began. Six new discs and new caps were placed on the flowers in an arrangement that was different from that in training. (For instance, if the flower had a blue disc (S+) during training, the flower would have a yellow disc (S-) during testing.) All flower caps were filled with water colored to look like honey, thus no reward and no scent was available during testing. A choice of a flower was recorded if a bee either hovered over the cap for two or more seconds, landed on the flower, and/or grasped the cap. As soon as a bee made a response, the experimenter recorded the color or pattern of the flower the bee chose for as long as the bee continued to visit flowers. Only the first six choices were analyzed because as the testing session with empty flowers progressed, there would be more opportunity for unlearning any discrimination.

Testing sessions lasted approximately one hour; after one hour bees no longer approached the flowers but remained stationary on the sides or ceiling of the flight cage. At the end of the testing session all discs and caps were removed from the flowers. The training discs and their caps were then placed on the flowers in the same arrangement as in testing. Bees were then allowed to train again until they were tested the following day. Testing followed by training continued in this fashion until a minimum of 100 responses was recorded for that condition (though only the first six choices by each bee were used). Testing lasted two to seven days.

**Training (Females)**. Training methods for the female bees were the same as for the males except for the following. During the training sessions the experimenter, using the gate in the corridor, would allow labelled bees to enter the flight cage. The experimenter would record the number of times each bee foraged from the S+ (rewarding) flowers, and allow the bees to return to the colony after they had foraged. Training sessions lasted 2-3 hours each. Worker bees were given a much shorter training period than the males because they visit many more flowers in a shorter period of time. Positions of the S+ and S- flowers were changed 2-3 times during a training session.

**Testing (Females)**. Testing methods for the female bees were the same as for the males except for the following. Bees were allowed to enter the flight cage one at a time. Bees were allowed to visit flowers for as long as possible but again, only the first six choices were analyzed. Once the bee began flying around the cage and ceased to approach the flowers, she was captured and returned to the colony. Testing sessions lasted approximately 2-3 hours.
Statistical Analysis. Because the data were counts with replication within individual bees, a replicated goodness-of-fit test with the G-statistic was used (Sokal and Rohlf 1981). In tests of significance, the G test-statistic is compared to a chi-square value. Two G-values were obtained: $G_H$ which tests for heterogeneity or individual differences, and $G_P$ which tests whether the pooled data (i.e., the group data) deviate from the expected proportion, which is a chance level of 50:50.

The analysis above could only detect deviations of group proportions from a theoretical value. A further analysis compared the four choice proportions in each of Figures 1-4. A logistic model which specifies a binomial error term was fit to the individual choice proportions using GLIM (Francis et al. 1993). We tested for the effect of sex, of stimulus assignment (for example whether yellow was the S+ and blue the S- or vice-versa), and their interaction.

RESULTS

A summary table of the analyses comparing each choice proportion to chance is given in Table 1.

Blue vs Yellow. For male and female bees the choice proportions and total number of observations for this condition are shown in Figure 1. Both the males and the females could discriminate between blue and yellow flowers significantly better than chance, both when the blue flowers were the S+ and the yellow flowers were the S- and vice-versa. All the $G$ values were significant for this discrimination. No significant individual differences were found (non-significant $G_H$; see Table 1).

The logistic analysis revealed no sex difference ($\chi^2 = 1.8; \text{df} = 1, \text{NS}$). The discrimination was better when the blue flowers were the S+ ($\chi^2 = 3.9; \text{df} = 1, p < .05$) though this effect was due to the poorer performance of the females when the yellow flowers were positive—the males performed at 98% accuracy in both stimulus assignments. The interaction between sex and stimulus assignment was not significant ($\chi^2 = 3.5; \text{df} = 1, \text{NS}$). No change between the first three and second three choices was detected ($\chi^2 = 0.1; \text{df} = 1, \text{NS}$) so the bees did not react to the absence of reward.

Two Radial Patterns (Sixths vs Quarters). Figure 2 displays the choice proportions and total number of observations. For the male bees, when the sixths pattern was the S+ and the quarters pattern was the S-, the choice proportions did not deviate significantly from chance ($G_H$ was nonsignificant; see Table 1). When the S+ and the S- were reversed, however, the S- (sixths pattern) was chosen significantly more often. There were no significant individual differences (nonsignificant $G_H$; see Table 1) and so this result can not be attributed to aberrant behavior on the part of a few bees compared to the rest of the group. For the females neither of the choice proportions differed significantly from chance nor were individual differences significant (Table 1).

The logistic analysis yielded no effect of sex ($\chi^2 = 3.22; \text{df} = 1, \text{NS}$), no effect of stimulus assignment ($\chi^2 = 2.10; \text{df} = 1, \text{NS}$), and no interaction ($\chi^2 = 2.11; \text{df} = 1, \text{NS}$). No change between the first three and second three choices was detected ($\chi^2 = 0.1; \text{df} = 1, \text{NS}$).

Concentric vs Sixths. The choice proportions and total number of observations for this condition are shown in Figure 3. Neither the males nor the females showed choice proportions which differed significantly from chance. For the females, however, both choice proportions were approximately equal to 60%, whereas for the males they were closer to 50%. Pooling the data across stimulus assignment revealed that the discrimination was significant for the females ($\chi^2 = 4.07; \text{df} = 1, p < .05$) but not for the males ($\chi^2 = .76; \text{df} = 1$). Individual differences were not significant (Table 1).
Table 1. Summary of G-Statistic Results for Each Testing Condition

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sex</th>
<th>df</th>
<th>(G_d)</th>
<th>df</th>
<th>(G_p)</th>
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</thead>
<tbody>
<tr>
<td>Blue vs. Yellow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>B (S+) vs. Y (S-)</td>
<td>M</td>
<td>9</td>
<td>4.27</td>
<td>1</td>
<td>55.48*</td>
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<td>F</td>
<td>7</td>
<td>4.31</td>
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<td>56.82*</td>
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<td>1</td>
<td>74.36*</td>
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<tr>
<td></td>
<td>F</td>
<td>7</td>
<td>5.00</td>
<td>1</td>
<td>32.14*</td>
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<tr>
<td>Sixths vs. Quarters</td>
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<td></td>
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<td>S (S+) vs. Q (S-)</td>
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<td>F</td>
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<td>0.36</td>
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<td>8.36</td>
<td>1</td>
<td>5.95</td>
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<tr>
<td></td>
<td>F</td>
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<td>1</td>
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<tr>
<td>Concentric vs. Sixths</td>
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<td></td>
<td></td>
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<tr>
<td>C (S+) vs. S (S-)</td>
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<td>4.68</td>
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<td>F</td>
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<td>8</td>
<td>6.88</td>
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<td>F</td>
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<td>4.37</td>
<td>1</td>
<td>1.60</td>
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<tr>
<td>Quarters vs. Concentric</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q (S+) vs. C (S-)</td>
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<td>3.93</td>
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<td>F</td>
<td>5</td>
<td>3.41</td>
<td>1</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Choices of S+:
* Above chance, \(p < .01\)
+ Below chance, \(p < .05\)
X When results from the two groups marked with an X were pooled, pooled results were above chance, \(p < .05\)

The logistic analysis showed that the sex difference in favor of females was significant \((\chi^2 = 4.16; \text{df} = 1, p < .05)\). The effect of stimulus assignment was not significant \((\chi^2 = 0; \text{df} = 1)\) and its interaction with sex was not significant either \((\chi^2 = .04; \text{df} = 1, \text{NS})\). No change was detected between the first three choices and the second three choices \((\chi^2 = .06; \text{df} = 1)\).

**Concentric vs Quarters.** Figure 4 displays the choice proportions and total number of observations for this condition. For males, when the quarters pattern was the S+ and the concentric pattern was the S-, the choice proportion did not differ from chance (Table 1). When the contingencies were reversed, however, a significant preference for the S- (the quarters) was found. Individual differences were not significant. For the females, the choice proportions did not differ from chance regardless of which pattern was the S+ and which pattern was the S-.

The logistic analysis did not reveal any sex difference \((\chi^2 = 2.75; \text{df} = 1)\) though an effect of stimulus assignment was detected \((\chi^2 = 4.85, \text{df} = 1, p < .05)\); choice proportions were higher when the quarters pattern was positive. The interaction between sex and stimulus assignment was not significant \((\chi^2 = 1.45; \text{df} = 1)\). No change between the first and second three choices was found \((\chi^2 = .77; \text{df} = 1)\).

In the pattern discriminations the G-tests and the logistic analyses might seem to yield conflicting results. In the first and third comparisons no sex difference was found even when one of the G-statistics was significant. The conflict, however, is only apparent. Notwithstanding the fact that the two kinds of analyses make different sorts of comparisons, whenever there was a significant effect, it always pointed to the same conclusion of superior performance by
the female bees. Indeed, pooling the data across the whole experiment, a significant sex difference was found ($\chi^2 = 7.6; df = 1, p < .01$) with a 10% advantage for the females (choice of the $S^+$ was, overall, 59% for males and 69% for females).

**DISCUSSION**

Male bees are uncooperative subjects in foraging studies: in our study they were much more “reluctant” to work than female bees. Nonetheless, the results on color discrimination validate the method: males could learn either that the blue flowers were rewarding or that the yellow flowers were rewarding. Indeed, the choice proportions could hardly have been higher: 98% in both cases. Of course, discrimination might have been less well achieved if colors other than blue and yellow had been used: blue and yellow artificial flowers elicit the shortest search times from bumble bees, at least for large flowers such as ours (Spaethe et al. 2001). Nonetheless, with respect to the primary objective of this paper, we can conclude that male bumble bees can attend to, learn and remember colors to discriminate between rewarding and unrewarding flowers. Moreover, their performance is comparable to that of workers. The same can not be said, however, for the pattern discriminations.

Three pairs of patterns were examined: (1) Radial Sixths vs Radial Quarters, (2) Concentric vs Radial Sixths, and (3) Radial Quarters vs Concentric. In the second pair, a significant sex difference was found: females showed choice proportions of about 60% regardless of which pattern was the $S^+$, and males did worse (Figure 3). For the other two comparisons, the patterns were not discriminated, and no sex difference was found. The discrimination failures might be attributable to insufficient statistical power and future research would benefit from larger samples (the results for the females in Figure 3 suggest twenty bees as a target figure for detecting a 60:40 discrimination). Pattern discriminations are indeed more difficult than color discriminations (Srinivasan 1994), especially if the flowers are presented horizontally rather than vertically. Males showed two choice proportions which were significantly below chance (Figures 2 and 4), and these remain a puzzle. If the bees could not learn a discrimination, choice proportions of chance would have been expected. If the bees had pronounced unlearned preferences, then a significant preference for a particular pattern should have been obtained not only when it was the $S^-$ but also when it was the $S^+$.

Overall the females outperformed males in pattern discriminations. From a functional point of view, this result confirmed our prediction that males would be less adept at floral discriminations because the cost of failure is only paid by the individual male and not by the brood which depend on females for nourishment. Although the data are suggestive and encourage further research, they most certainly do not justify any conclusions regarding possible sex differences in cognition such as attention, pattern recognition, associative learning and/or memory. Further research might show, for example, that the relatively poor performance of the males was due to a self-inflicted impoverishment of the learning conditions. The males were trained for longer periods of time because they were generally less active, and so in terms of opportunity for learning, our procedure was biased in favor of the males, but the numbers of exposures to the $S^+$ and to the $S^-$ were not equated between the sexes. Even if they could be, the longer intervals in between flower visits for the males would provide more opportunity for forgetting. Finding a means of equating the learning conditions would be one of many steps in undertaking further male-female comparisons for which the present study has set the stage. One common practice which might be used in future research is to train the bees to asymptotic performance. This method, however, does not necessarily equate learning for males and females for two reasons: (1) such learning would likely take longer time and more floral encounters for the males (2) asymptotic performance is by definition a ceiling...
effect and comparisons under those conditions are usually inadvisable. Future research should also include replication between colonies. In this study males came from one colony and workers from another. Although our previous research (Simonds and Plowright, unpublished data) found no colony differences in unlearned color and pattern preferences, we cannot rule out the possibility of colony differences in color and pattern learning ability.

One possible objection to this study is in the use of honey solution rather than odorless sugar solution during training. During training, bees had the opportunity to learn either scent, or pattern/color, or both odor and visual characteristics to discriminate the rewarding from the unrewarding flowers. During testing, however, water instead of honey solution was used and so only pattern/color was available to make discriminations, but not scent. The strong discrimination for the Blue vs. Yellow condition (Figure 1) shows that color was learned in training, regardless of whether scent may also have been used during training. The same can be said for the weak but significant discrimination by the workers in the Concentric vs Radial Sixths condition: the bees must have learned the association between reward and visual pattern. Indeed, our previous work (Korneluk and Plowright 1995, Plowright 1997, Plowright et al. 2001) which used honey solution in training [bumble bees are reticent to land on artificial flowers in the absence of scent (Lunau, Wacht and Chittka 1996)] and water in testing has also shown successful pattern learning and recognition by bumble bees, so the use of honey solution per se is not a concern when pattern discriminations are obtained. More problematic, however, are the conditions where no pattern discrimination was obtained: it is possible that patterns were not learned in training but instead scent may have been used by the bees to discriminate between the rewarding and unrewarding flowers. Perhaps when bees are presented with some complex visual discriminations in training, they then resort to using the cue of scent when foraging and mostly ignore the patterns on the flowers. This interpretation does not undermine the conclusion that some pattern discriminations are particularly difficult, and perhaps especially difficult for males. It does however suggest a possible way of forcing bees to make a discrimination which they otherwise would not make.

Labor division in insect societies is anything but equal, and our results highlight a behavioral correlate of the general rule that female bees work and males don't: females showed better performance in floral pattern discriminations, though further research is needed to delineate the conditions under which this might occur. Males are quite capable, however, of learning color discriminations between rewarding and unrewarding flowers. The sociobiology of male idleness has been well worked out (Hamilton 1964) but its psychology remains to be elucidated.

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LITERATURE CITED


