Title:

Shift work dynamics and division of labor: honeybee foraging and fanning tasks

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1   **Abstract:**

2   In complex societies common social needs such as vigilance, care giving, resource gathering,

3   and production are attended around the clock. In humans, these services are constantly

4   provided using a shift work strategy where different individuals, or groups of individuals

5   perform their tasks at different times of the day. However, shift work strategy in job

6   organization in other social organisms remains unclear. Previous studies in honeybees for two

7   jobs support shift work for only pollen foragers and not for nursing behavior. Here we examined

8   shift work dynamics for three types of jobs performed by honeybee foragers. Specifically, we

9   studied pollen foragers, non-pollen foragers and bees fanning at the entrance of the colony, a

10 job important for orientation and temperature control. Major features of the observed shift work

11 were: 1) individuals can be divided into early and late shifts; 2) there are constant workers; 3)

12 based on job, shift work is performed by fewer or greater number of individuals; 4) shift work

13 of an individual is plastic and may change with age; 5) foraging and fanning shifts are coupled

14 yet dissociable. This study adds to the findings that shift work is not exclusive to modern

15 human societies and that a natural form of shift work exists in honeybees. These results

16 suggest that shift work in honeybees is a feature of worker division of labor. Future studies

17 aiming to further understand the structure, function and mechanism of this natural form of shift

18 work in honey bees not only could have an impact on agriculture but also may provide insight

19 into alternative forms of shift work strategies that may reduce the various health problems

20 associated with shift work in humans.

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Introduction:

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29   Principles that organize social work are common across social organisms (Gordon,

30   2007; Oster & Wilson, 1978). Specialization, based on ontogenetic, morphological or genetic

31   mechanisms, occurs in many social species (Fjerdingstad & Crozier, 2006; Jeanson, Fewell,

32   Gorelick, & Bertram, 2007; O'Riain, Jarvis, Alexander, Buffenstein, & Peeters, 2000;

33   Robinson, 1992). Parallel processes performed by multiple agents result in networks.

34   Networks of individuals can modulate behavior via feedback regulation, that may depend on

35   order of task performance, such as foraging followed by nectar unloading and storage (Craig

36   et al., 2012; Jeanne, 1986; O'Donnell & Jeanne, 1992) or based on chemical communication

37   such as pheromones or cuticular hydrocarbons (Inoshita, Martin, Marion-Poll, & Ferveur,

38   2011; Pankiw, 2004; Sagili, Pankiw, & Metz, 2011). Spatial organization confines activities to

39   specific locations, often enhancing the effects of other mechanisms that organize work (Jandt

40   & Dornhaus, 2009; Mersch, Crespi, & Keller, 2013). Temporal organization, restricts the

41   performance of a task to a specific time period of the day and may have molecular, cellular

42   and behavioral correlates (C S Pittendrigh, 1993; Roenneberg, Wirz-Justice, & Merrow, 2003;

43   Southerton, 2006). At the behavioral level, various temporal organization strategies have

44   emerged throughout history. Among them, shift work strategies have become a mainstay in

45   modern human societies (Folkard, 2003a; Pati, Chandrawanshi, & Reinberg, 2001). However,

46   shift work has not been studied extensively in social insects.

47   Shift work is a method of organizing individuals or groups to perform specific tasks at

48   different times of the day such that these tasks can be continuously performed (IARC Working

49   Group on the Evaluation of Carcinogenic Risks to Humans, 2010; Pati et al., 2001).

50   Professions such as health care, emergency response teams (e.g. firemen), transportation

51   and food service use various shift work strategies to provide these essential services around

52   the clock (Adan et al., 2012). Although shift work strategies succeed by providing many

53   benefits for society and employers, there are costs at the individual and social level.

54   Catastrophes such as the Chernobyl nuclear meltdown, Three Mile Island and the Exxon

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55   Valdez oil spill, have been linked to errors associated with shift work (Akerstedt & Wright,

56   2009; Folkard, 2003b; Klerman, 2005; Mitler et al., 1988; Pati et al., 2001; S. M W Rajaratnam

57   & Arendt, 2001). Studies examining the relationship of shift work and health problems provide

58   convincing evidence that misalignment of circadian rhythms is one of the key contributors to

59   many, if not all, of the negative effects associated with shift work (Knutsson, 1989; Shantha M

60   W Rajaratnam, Howard, & Grunstein, 2013). It has thus become important to study what

61   strategies other social organisms, use to achieve their 24/7 needs.

62   In honeybees, colony structure is best defined by castes with clear division of

63   labor system with diploid queens and haploid males (drones) attending reproductive tasks,

64   while sterile diploid workers perform all other jobs associated with colony maintenance

65   (Robinson, 1992; Mark L Winston, 1987). Within workers, division of labor is an age-related

66   process, where workers perform a series of tasks from the moment they emerge as adults

67   and change tasks as they age until they begin foraging (~21 days of age) (Seeley, 1985, 1995;

68   Mark L Winston, 1987). The rate of division of labor in workers has been shown to be

69   genetically, behaviorally and hormonally regulated and as a result, individuals of the same

70   age can be observed performing different tasks (Giray & Robinson, 1996; Giray, Guzman-

71   Novoa, et al., 1999; Huang & Robinson, 1992; Leoncini et al., 2004).

72   In the colony tasks such as brood care, cleaning cells, fanning and foraging are

73   performed throughout the day, or for extended periods of time. Whether individuals are

74   constantly performing these tasks or if they use shift work strategies has been asked. Previous

75   work examined if nurses used shift work or similar strategies to take care of the brood. Moore

76   and colleagues (1998) marked and observed task performance of individual bees and found

77   that brood care is performed throughout the day without specific timing (Moore, Angel,

78   Cheeseman, Fahrbach, & Robinson, 1998). This coincides with the idea that the brood

79   releases pheromones that make honey bee workers lose their circadian rhythmicity such that

80   they feed the brood (Moore, 2001; Nijland & Hepburn, 1985; Yair Shemesh, Eban-Rothschild,

81   Cohen, & Bloch, 2010; Spangler, 1972; Stussi, 1972). Based on these studies nurses take

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82   care and feed the brood, in a similar manner as human mothers take care of new-born children,

83   around the clock. However, this finding in brood care may not extend to other jobs in the

84   honeybee colony.

85   Fanning behavior is a task that workers perform to regulate the temperature of the

86   colony, release Nasonov's pheromone and mature honey (Seeley, 1995; Mark L Winston,

87   1987). A study examining thermoregulation of the colony, focusing of fanning behavior, found

88   that colonies with a uniform genetic background (originated from one male) are less efficient

89   at maintaining temperature levels compared to colonies with a diverse genetic background (J

90   C Jones, Myerscough, Graham, & Oldroyd, 2004). However, whether bees use a particular

91   strategy to organize fanning throughout the day has remained unexplored.

92   In the case of foraging, bees use the full daylight period in order to gather the various

93   resources that colonies need on a daily basis. Through the use of sun compass navigation (R

94   M Goodwin & Lewis, 1987; von Frisch, 1967), time memory (Moore & Doherty, 2009; Moore,

95   Van Nest, & Seier, 2011; B. N. Van Nest & Moore, 2012) and circadian rhythms(Bloch &

96   Robinson, 2001; Cheeseman et al., 2012; Eban-Rothschild & Bloch, 2012; Y Shemesh, Cohen,

97   & Bloch, 2007; Yair Shemesh et al., 2010), bees predict the availability of different resources

98   throughout the day. Individual workers can specialize in the collection of a specific resource

99   such as pollen, nectar or water (Fewell & Page, 1993; Robinson & Page, 1989; Seeley, 1995).

100 Studies examining the underlying factors of this resource specialization have found genetic,

101 neuroendocrine and behavioral differences between pollen and nectar specialists (Barron,

102 Maleszka, Vander Meer, & Robinson, 2007; Erber, Hoormann, & Scheiner, 2006; Giray,

103 Galindo-Cardona, & Oskay, 2007; Page & Erber, 2002; Scheiner, Barnert, & Erber, 2003;

104 Scheiner, Page, & Erber, 2001; Scheiner, Plückhahn, Oney, Blenau, & Erber, 2002; Scheiner,

105 Toteva, Reim, SÃ¸vik, & Barron, 2014; Taylor, Robinson, Logan, Laverty, & Mercer, 1992;

106 Wagener-Hulme, Kuehn, Schulz, & Robinson, 1999).

107 In contrast to brood care, in a recent study, researchers captured incoming pollen

108   foragers in the morning and afternoon for four days and genotyped them with microsatellite

109   DNA markers (Kraus, Gerecke, & Moritz, 2011). They uncovered that a small percentage of

110   pollen foragers from specific patrilines were only captured in the morning, while foragers from

111   other patrilines were only captured in the afternoon. This finding suggests that some pollen

112   foragers make their foraging trips in shifts and this behavior is in part influenced by the genetic

113   origin of the individual (Kraus et al., 2011). Organization of shift work for pollen foraging and

114   potentially other jobs can be examined through direct observations as was done for nursing.

115   Here we present a comprehensive analysis of foraging and fanning behavior in

116   honeybee workers to determine the presence and organization of shift work. In this study, we

117   1) determined whether a shift work strategy is evident in pollen, non-pollen foraging and

118   behaviors and if so, 2) describe the behavioral characteristics of this shift work. We performed

119   direct behavioral observations at the hive entrance workers of an age cohort. Our central

120   hypothesis was that if foragers perform specific tasks in shifts then we would observe groups

121   of individuals performing these behaviors at specific times of the day. To address specific

122   characteristics of shift work we conducted our observations over most of the foraging life of

123   the age cohort. In this way, we could examine the degree of plasticity associated with shift

124   work and whether the organization of shift workers varies between different jobs. Lastly, we

125   examined possible relationships of the temporal allocation between foraging and fanning tasks

126   for each individual.

127   **Materials and Methods**

128   1. Observation ramp

129   A two-story hive with a naturally mated queen was fitted with an extended entrance ramp

130   with a glass top measuring 45cm wide and 40cm in length (Giray et al., 2007). Briefly, to train

131   the bees to the entrance ramp, we first installed the ramp without the glass top. Two days

132   following the placement of the ramp a piece of glass of 5cm in length was lined with colored

133   tape and placed in the ramp. The following days the length of the glass was slowly extended

134   until it covered the full length of the ramp. The glass top assured a narrow space within which

135   bees were unable to cover each other or walk upside down.

2. Bees

137   Honeybee workers were obtained from 2 healthy colonies (collection colonies) with a

138   naturally mated queen at the University of Puerto Rico Bee Research Facility at the Gurabo

139   Experimental Agriculture Station. From each of the colonies, we marked three groups of 500

140   bees (n=3,000 marked individuals) with a three-day interval between each marking group. To

141   mark, we extracted 2 brood frames with large numbers of capped brood in the afternoon. The

142   frames were gently brushed to remove the attending nurses and transported to our laboratory

143   incubator (Thermo Scientific Precision Incubator 815), where they remained overnight. Bees

144   that had emerged on the following morning were extracted and individualized by applying a

145   colored numbered tag in the thorax and a paint dot (acrylic, Testors[®]:   TES1127TT,

146   TES1146TT and TES1172TT) in the abdomen identifying the Age cohort. After marking, bees

147   were placed inside of the colony that had been previously fitted with the observation ramp.

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3. Observation periods

150   Observations were twice a day for 14 days, from 9:00-11:00 and from 14:00-16:00, in a

151   similar manner as in a previous study (Krauss et al., 2011). In addition, these periods were

152   chosen to prevent the overlap of foraging trips between observation periods. Researchers

153   have observed that the duration of foraging trips can range from 4-25 min on average but

154   foraging trips longer than 50 minutes have been recorded ((Mattu, Raj, & Thakur, 2012; Partap,

155   Shukla, & Verma, 2000; Singh, 2009; Wagner, Van Nest, Hobbs, & Moore, 2013). Before each

156   observation period began, a thin coating of petroleum jelly (Vaseline[®]) was applied to the glass

157   to prevent bees from walking upside down. Colonies were observed sequentially during the

158   summer, in this manner colony 1 observations took place from May 25th – June 7th, 2012 while

159   colony 2 observations took place from June 28th – July 11th, 2012. During observation the

160   entry, exit and fanning behavior of each individual was recorded in a laboratory notebook with

161   an accompanying time stamp, and later transcribed to JMP for data analysis.

162   During the 14-day observation periods, for colony 1, of the 1,500 marked individuals we

163   were able to observe a total of 1,030 bees and recorded 5,102 individual observations. For

164   the same duration, 535 of 1,500 marked bees were observed in colony 2 and a total of 2,698

165   individual observations were recorded. Observations for colony 2 took place during Puerto

166   Rico's rainy season, and constant interruptions due to weather conditions may account for the

167   differences in the number of observations. Our methodology allowed us to record, on

168   average5 direct behavioral observation for each of more than a thousand individuals.

169   4. Morningness ratio

170   To establish if bees perform shift work for each of the observed behaviors (foraging trips

171   or fanning) we tabulated the number of observations during the morning observation periods

172   and afternoon observation periods for each individual. We then calculated the ratio of morning

173   observations over the total observations. This formula was modified from that previously

174   described and used by Moore et al. (1998). In this manner, individuals that mainly forage or

175   fan in the afternoon would have morningness ratios close to 0 (afternoon shift), while those

176   that forage or fan mainly in the morning would have a ratio close to 1 (morning shift). Similarly,

177   if individuals have no temporal preference for performing a specific task, they would have a

178   ratio close to 0.5 (no shift).

179   5. Foraging patterns

180   To answer if bees' preference to forage in the morning or afternoon changed as they aged,

181   we examined each individual's foraging trip observations in scatterplots. We identified five

182   foraging pattern phenotypes: morning; afternoon; morning-afternoon, who began in the

183   morning and after some time switched to the afternoon; afternoon-morning, began in the

184   afternoon and switched in the morning; and constant. For an individual to be included in a

185   foraging pattern their observations had to span for a period of 6 days or more and the majority

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186   of these had to have occurred within the 12-19 days of age to control for any possible bias

187   fewer observations on an individual may generate.

188   6.   Data Analysis

189   For both foraging trips and fanning behavior only individuals with 3 or more observations

190   were considered for data analysis. We also excluded individuals for whom all observations

191   were taken on the same day. Comparison of the observed frequency distributions of the

192   morningness ratio for foraging trips and fanning behavior, for each colony, was statistically

193   compared using chi-square goodness of fit with theoretical frequencies from a binomial

194   distribution that assumes no shift work (null hypothesis). To compare the observed

195   distributions of each colony (foraging trips or fanning) we utilized the Kolmogorov-Smirnov test

196   of distributions. Median test was used to compare the foraging pattern frequency distributions,

197   the mean trips taken, the probability of taking a foraging trip and the mean number of trips in

198   a foraging period. For the correlations of the foraging and fanning morningness ratios, pairs

199   of observations from the same day were tested with Kendall's tau association test. All

200   statistical analyses were performed using the statistical software program JMP (SAS Institute

201   Inc.). Figures were prepared using GraphPad PRISM 6.00, GraphPad software, La Jolla

202   California USA and R (R Core Team).

203   **Results:**

204   ***Foragers use two temporal strategies to gather resources for the hive.***

205   To determine if all foragers go out throughout the day or if groups of individual

206   bees forage at different times of the day, we calculated the number of morning observations

207   over the total number of observations (morningness ratio) for each forager. Since foraging in

208   African-hybrids, such as the ones used in this study, can start as early as 11 days of age

209   (Giray, Huang, Guzman-Novoa, & Robinson, 1999; M L Winston, 2003; Mark L Winston, 1987),

210   we used the data observations from 17 days of age onward. Consistent with our hypothesis,

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211   our results revealed that more than 40% of the individuals exclusively foraged either in the

212   morning or afternoon, now on referred to as shift workers (Figure 1A). In addition, to shift

213   workers, we also observed constant workers, which foraged both in the morning and afternoon.

214   To determine if the observed shift work ratios were significantly different from chance, the

215   observed distribution was compared with a theoretical binomial distribution that assumed the

216   absence of shifts. This comparison using Pearson's X^2 resulted in significant differences for

217   both colonies sampled, suggesting that groups of workers forage at different times of the day

218   (colony 1: Pearson's $X^2= 1009.53$, $p<< 0.01$, n=227; colony 2: Pearson's $X^2= 647.73$, $p <<$

219   0.01, n=142). Statistical comparison using the Kolmogorov-Smirnov statistical analysis was

220   also performed to compare the observed distributions of the sampled colonies. This resulted

221   in significant differences between the observed distributions of each colony (Kolmogorov-

222   Smirnov two-sided test, D=0.1671, $p=0.02$), suggesting possible colony-colony differences in

223   shift work.

224   Further examination of our data set revealed that the number of observations between

225   shift workers and constant workers varied greatly. We hypothesized that constant workers,

226   who forage throughout the day, would perform at least twice the foraging trips than shift

227   workers, who only forage at specific times of the day. To test this hypothesis, we took into

228   account the number of observation periods, that constant workers would be observed in both

229   periods and the proportion of constant workers that were observed we predicted that constant

230   workers would be responsible for ~75% of the observed foraging trips. Consistent with our

231   prediction, constant workers account for more than 80% of our observed foraging trips, while

232   shift workers performed less than 20% of foraging trips observed (Figure 1B).
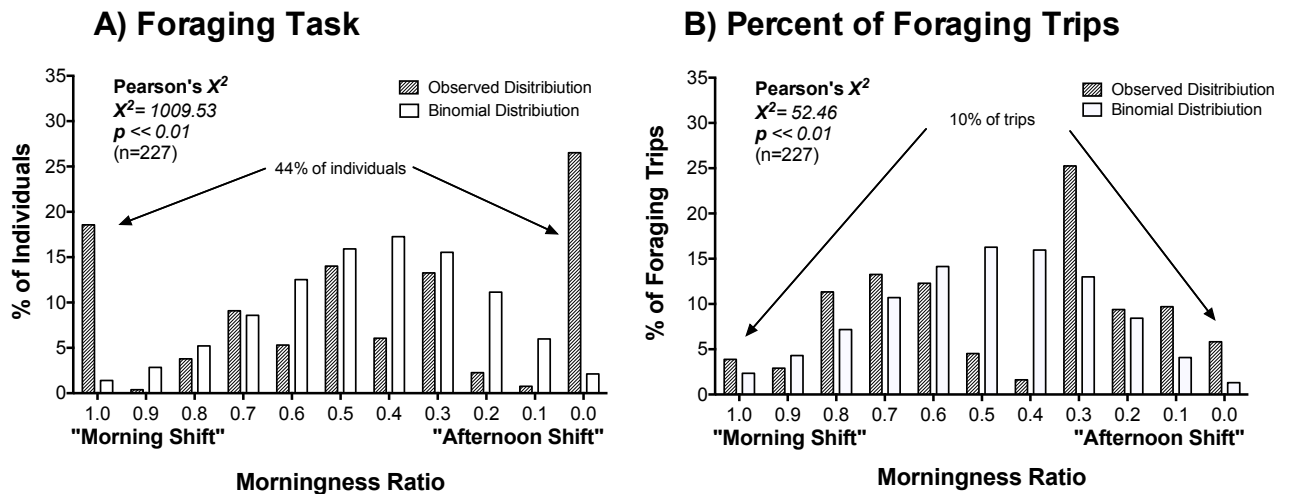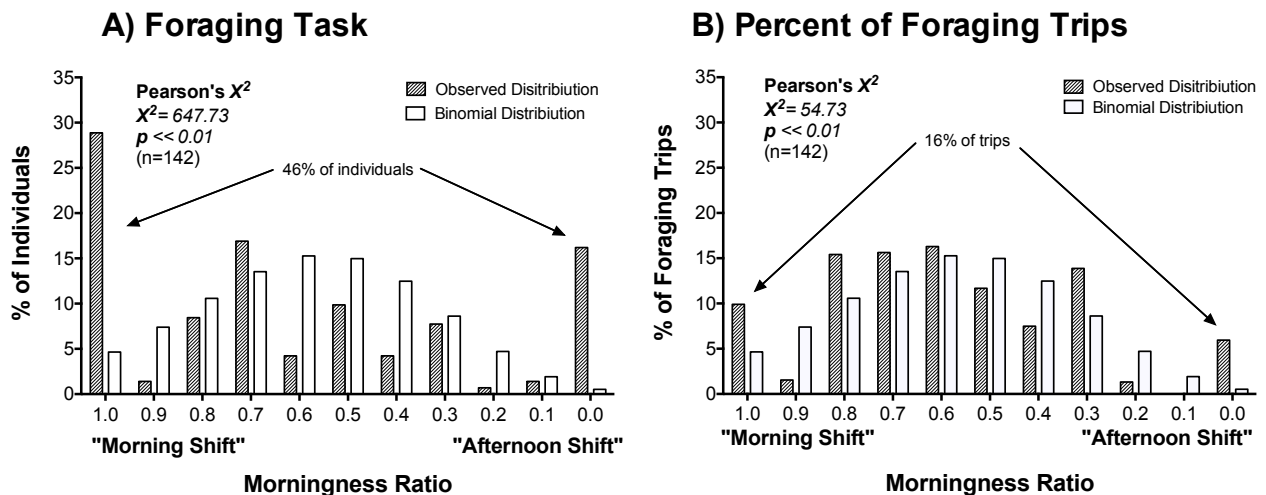
Figure 1. **Exclusive shifts in morning and afternoon are present foraging task. (A)** Frequency distribution of observed morningness ratio for per cent of individuals (shaded bars) compared to a theoretical binomial distribution (white bars) revealed that more than 40% of sampled individuals foraged exclusively in the morning or afternoon as pointed as pointed by arrows for **1)** colony 1 and **2)** colony 2. Goodness of fit test revealed significant differences between the observed and theoretical distributions (colony 1: Pearson's $X^2 = 1009.53$, $p << 0.01$, n=227; colony 2: Pearson's $X^2 = 647.73$, $p << 0.01$, n=142). Comparison between the observed distributions of individuals for each colony via Kolmogorov-Smirnov two-tailed test revealed significant differences between the observed morningness ratio distributions (D=0.17, $p=0.02$). **(B)** Frequency distribution of morningness ratio and the present of trips observed (shaded bars) reveals that less than 20% of trips are made by foragers who exclusively forage in the morning or afternoon as pointed. Comparison of each of the observed distribution with a theoretical binomial distribution (white bars) revealed significant differences between the observed and theoretical distributions (colony 1: Pearson's $X^2 = 52.46$, $p << 0.01$, n=227; colony 2: Pearson's $X^2 = 54.73$, $p << 0.01$, n=142). Comparison between the per cent of trips for each colony via the Kolmogorov-Smirnov two-tailed test revealed significant differences each observed distribution (D=0.31, p<<0.01).

251   ***Shift workers within pollen foragers represent a small subset of individuals***

252   Previous work presenting genetic evidence for shift work in foragers was restricted to

253   pollen foragers (Kraus et al., 2011). In our experiments we observed marked foragers in

254   general and were able to discern between pollen and non-pollen foragers. By separating

255   pollen and non-pollen foragers we found within both pollen and non-pollen foragers there are

256   individuals foraging in shifts (Figure 2). In the case of pollen foragers less than 10% perform

257   foraging in shifts, which is consistent to the genetic work previously published (Kraus et al.,

258   2011). Conversely, 36% percept of non-pollen individuals exclusively forage in the morning or

259   afternoon (Figure 2), suggesting that non-pollen foraging has a stronger shift worker
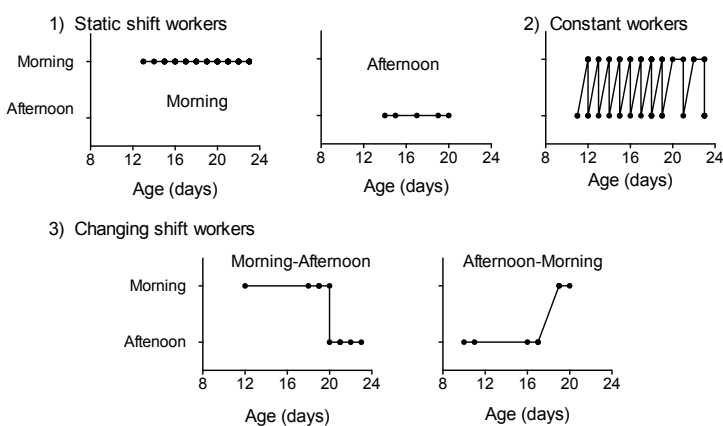
260   component than pollen foraging.
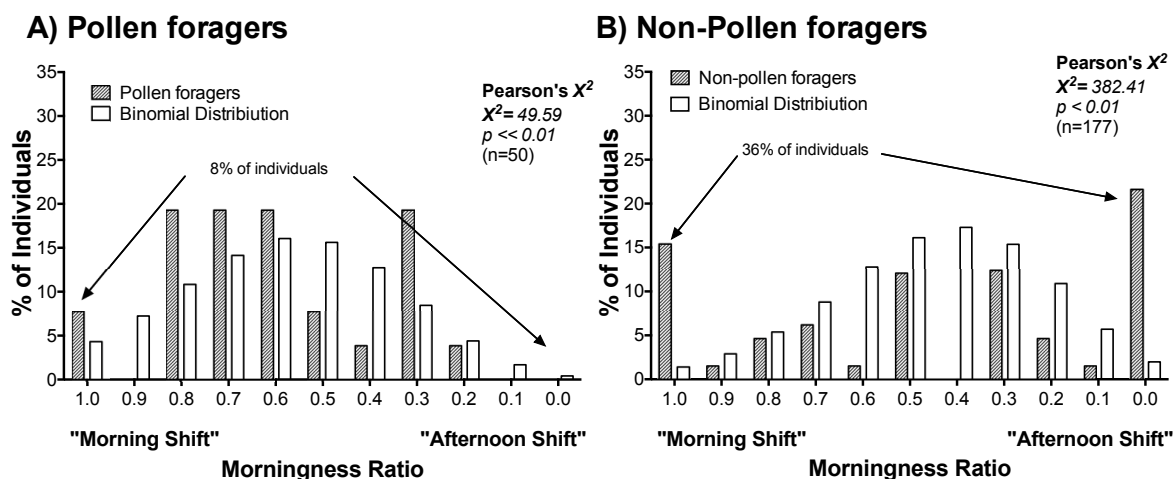
261

262   Figure 2. **Shift work allocation depends on foraging specialization were only 8% of pollen**
263   **foragers present a shift. A)** Frequency distribution of observed morningness ratio for pollen
264   specialists of colony 1 (shaded bars) compared to a theoretical binomial distribution (white bars)
265   of the null hypothesis. Goodness of fit test reveals significant differences between the observed
266   and theoretical distributions (Pearson's X^2= 49.59, p<< 0.01, n=50). **B)** Frequency distribution of
267   observed morningness ratio of non-pollen specialists of colony 1 (shaded bars) compared to a
268   theoretical binomial distribution (white bars) of the null hypothesis (Pearson's X^2= 382.41, p<< 0.01,
269   n=117). Pollen specialists compose less than 20% of individuals that perform foraging exclusively
270   in the morning or afternoon

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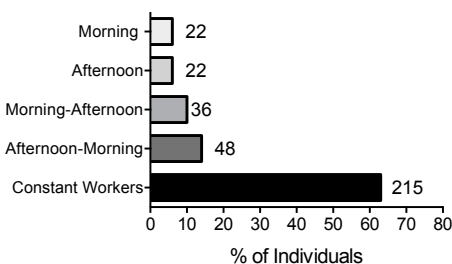272   ***Foraging shifts may change as bees age***

273   Since division of labor in honey bee workers is a complex age based process (Seeley,

274   1985, 1995; Mark L Winston, 1987), we hypothesized that age-related plasticity may be

275   evident in worker shifts. Our approach to address this interest was to examine those

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276   individuals for which data was collected over a 6-day time period. Our analysis described five

277   distinct behavioral patterns, which we sorted into different groups: 1) individuals that preferred

278   to forage during one of the periods (morning or afternoon), classified as static shift workers,

279   and 2) individuals that foraged indiscriminately in either period, classified as constant workers

280   (Figure 3 A). In addition, a third foraging pattern was observed, where individuals presented

281   a shift and after some time changed from that shift to the opposite and classified as changing

282   shift workers (morning-afternoon, afternoon-morning) (Figure 3A). Comparing the frequency

283   of each of the foraging patterns shows that constant workers represent more than 60% of the

284   observed foraging population (Figure 3B). We further studied individuals who changed shifts

285   to establish if there was a specific time window in the forager's life for this change and whether

286   the nature of this change in shift is endogenous or exogenous in origin. By establishing the

287   age at which each of the observed individuals changed shift we were able to establish the age

288   range that presents the highest probability a forager changes shift (Figure 3C). Our results

289   revealed that approximately 75% of individuals change shifts from 11-19 days of age, the early
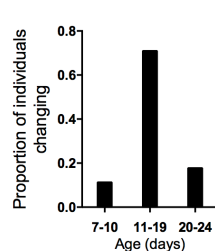
290   stage of the individuals foraging life.

Figure 3. **Shift work in foraging is plastic and can change with age.** **A)** Examples of the 5 foraging patterns obtained from honeybee entry and exit data from entrance observations: 1) Static Shifts (Morning and Afternoon); 2) Changing shifts (Morning-Afternoon and Afternoon-Morning); and 3) Constant foragers. **B)** Proportion of individuals changing shifts (morning to afternoon or afternoon to morning) at different age blocks. No significant differences were found between the colonies. **C)** Foraging pattern distribution of sampled individuals. Non selective individuals makeup more than 60% of our sample group, while approximately 20-25% of individuals change shifts once during their life and around 15% of individuals have static shifts. Comparison between colonies via contingency analysis did not reveal significant differences.

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316   *Fanning is performed in shifts*

317   While our results show that foraging is performed in shifts in honeybee colonies,

318   whether the observed shift work is endogenously driven or regulated by environmental factors,

319   such as flowers, was not distinguishable in our data set. For this reason, we analyzed if fanning

320   behavior at the entrance of the colony was done in shifts. Given the narrow regulation of

321   temperature in honeybee colonies we hypothesized that fanning behavior at the entrance of

322   the colony would be performed by some individuals in shifts and by others constantly

323   throughout the day. Consistent with this hypothesis, our results show that some workers

324   perform fanning in shifts, while others were observed fanning throughout the day (Figure 4).

325   Comparison between the theoretical binomial distribution for no shift work and the observed

326   distribution via Pearson's X^2 resulted in significant differences for both colonies sampled

327   (colony 1: $X^2 = 258.91, p < 0.001, n=45$; colony 2: $X^2 = 529.69\ p < 0.001, n=22$; Figure 4). In

328   addition, comparison between colonies via Kolmogorov-Smirnov test resulted in significant

329   differences between the observed distributions for the colonies ($D = 0.346, p \leq 0.05$). The finding

330   that fanning is also performed in shifts and colonies differ in distribution of individuals, suggest that
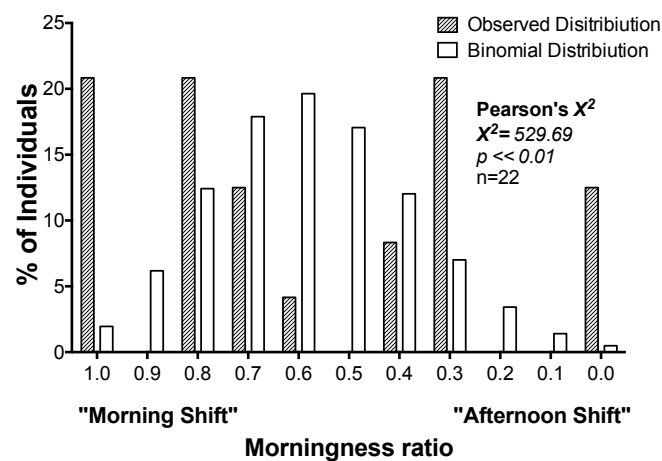
331   shift work may be endogenously driven.

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A) Colony 1

Fanning Task
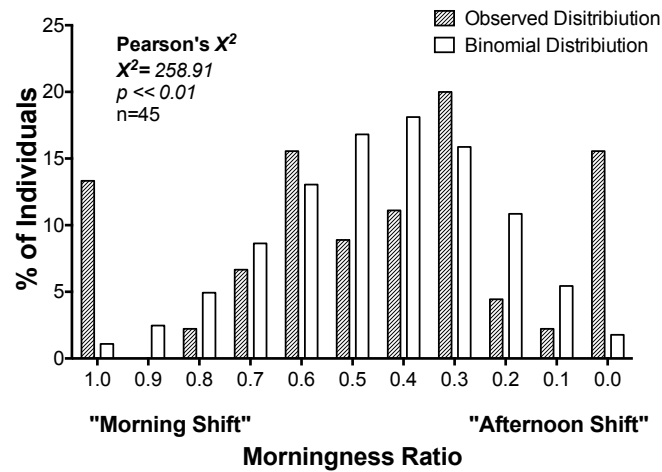
B) Colony 2

Fanning Task

Figure 4. **Exclusive morning and afternoon shifts are present in fanning task. A)** Frequency distribution of the observed morningness ratio for colony 1 (shaded bars) of fanning behavior in the observation ramp. The observed distribution was compared to a theoretical binomial distribution (white bars) that assumes no shift work. Goodness of fit test revealed significant differences between the observed and theoretical distributions ($X^2 = 258.91$, $p<<0.01$, n=45). **B)** Frequency distributions of the observed morningness ratio (shaded bars) and theoretical binomial distribution (white bars for fanning behavior of colony 2. Consistent with the result from colony 1, Goodness of fit test showed significant differences between the observed and the binomial distribution ($X^2=529.69$, $p<<0.01$, n=22). Comparison between the observed distributions for fanning behavior via Kolmogorov-Smirnov two-tailed test revealed significant differences between the frequency distributions of each colony (D= 0.346, $p< 0.05$).
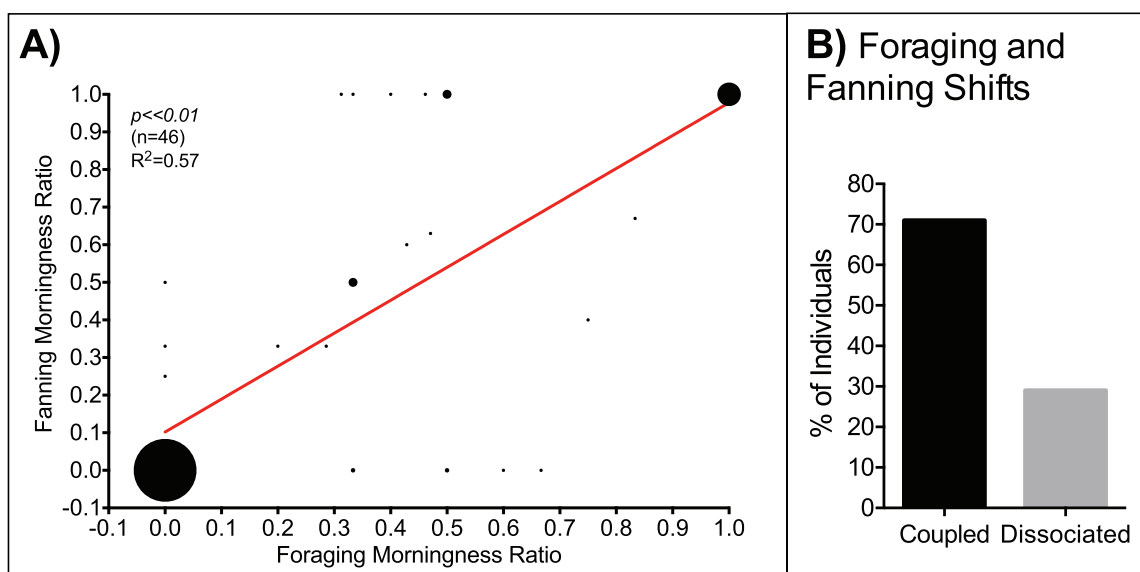
Endogenous relationship of foraging and fanning shifts

To examine how foraging and fanning shifts may be related we compared foraging and

fanning morningness ratio of individuals that performed both foraging and fanning during our

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348   observations. This analysis resulted in a positive correlation between foraging and fanning shifts,

349   suggesting that shift in one behavior influences the shift in the other (Figure 5A). However, upon

350   closer inspection we observed that there were individuals had a shift for foraging but not for fanning

351   and vice versa. Individuals that present shifts in both foraging and fanning behavior or lacked shifts

352   were classified as presenting a non-dissociable shift, while individuals with shift in either foraging

353   or fanning behavior were classified as dissociable shifts. By doing this we found that ~30% of

354   individuals present a dissociable shift (Figure 5B). These results suggest that foraging and fanning

355   shifts are processes that are connected yet dissociable.

356

357   **Figure 5. Foraging and fanning shifts are coupled yet dissociable behaviors. A)**
358   Pearson correlation of foraging and fanning morningness ratios for individuals that performed
359   both tasks resulted in a positive correlation ($R^2=0.57$, $p<<0.01$, n=46). The size of the dots is
360   representative of the number of individuals in each data point. **B)** Per cent of individuals who's
361   foraging and fanning correlates (coupled) compared with those that do not correlate
362   (dissociated).

363

364   **Discussion:**

365   The most significant finding of this study is that different shift work strategies contribute

366   to the organization of different jobs in the honeybee colony. Foraging bees take advantage of

367   the full daylight period to collect resources for the colony (Moore & Doherty, 2009; Moore &

368   Rankin, 1983; Byron N. Van Nest & Moore, 2012; von Frisch, 1967; Wagner et al., 2013; Mark

16

369   L Winston, 1987). Before our findings, it was not clear if foraging was performed continuously

370   by each individual throughout the day or if distinct sub-groups (shifts) foraged at different times

371   of the day. Here we show that both of these strategies are present in honey bee colonies, i.e.

372   there are foragers that constantly work throughout the daylight period (Constant workers) and

373   groups of foragers that only work in the morning or the afternoon (Shift workers) (Figure 1). In

374   addition, we characterize various features of the honeybee shift work strategy. We observed

375   that the demography of shift workers varies from task to task (Figure 2) and can be divided

376   into individuals that maintain the same shift as they age, and those that change from one shift

377   period to another (Figure 3). We also show that fanning, another task performed by workers,

378   has a similar composition, with some individuals performing the job constantly throughout the

379   day, and others doing so in shifts (Figure 4). Interestingly, around 60% of individuals, that were

380   observed foraging and fanning, showed the same shift for both behaviors (Figure 5),

381   suggesting that the shifts are coupled yet dissociable from one another.

382   We found that more than 40% of the individuals perform foraging trips exclusively in

383   either the morning or the afternoon, while the remaining individuals (constant workers) forage

384   throughout the daylight period (Figure 1). A previous study exploring the temporal organization

385   of brood care found that nurses work around the clock (Moore et al., 1998). Their finding is

386   consistent with the lack of circadian rhythmicity of nurses in the colony and the constant

387   demand of brood care, regulated by brood pheromones (Yair Shemesh et al., 2010). In

388   contrast, foragers are thought to rely on their circadian rhythms and time memory to

389   successfully collect different resources and return to the colony (Moore & Doherty, 2009;

390   Byron N. Van Nest & Moore, 2012; Wagner et al., 2013). The presence of both types of

391   foragers (constant, shift workers) may be adaptive to the colony, and it could potentially result

392   in the daylight period being more efficiently utilized by foragers.

393   Consistent with our hypothesis that the majority of foraging would be performed by

394   constant workers, our results show that constant workers perform the majority (~80%) of

395   foraging trips (Figure 1). We expected that constant workers would perform at least twice the

396   number of foraging trips than shift workers. By taking into account that we had 2 observation

397   periods, that constant workers will be observed at both periods, and the proportion of shift

398   workers and constant workers in our sample, we expected that constant workers would

399   perform ~75% of foraging trips. Since the predicted proportion of trips was similar to the

400   predicted value (75% predicted vs. 80% obtained), the observed differences in workload

401   between shift workers and constant workers can be accredited to 1) the higher proportion of

402   constant workers and 2) the two potential observation periods for constant workers. It is likely

403   that shift work was not uncovered directly until now since the majority of studies examining

404   foragers at the colony or in artificial feeders make observations throughout the day, and until

405   recently did not identify each individual. This combined with the low percentage of foraging

406   flights taken by shift workers would significantly reduce the probability of collecting and

407   observing shift workers in previous experimental setups.

408   Since honey bee foragers match their foraging activity to the time when the resource

409   they are collecting is at the peak of production and establish a time memory of this event that

410   allows them to anticipate resource availability (Moore & Doherty, 2009; Moore & Rankin, 1983;

411   Moore, Siegfried, Wilson, & Rankin, 1989; Moore et al., 2011; Byron N. Van Nest & Moore,

412   2012; Wagner et al., 2013), it is possible that shift workers and constant workers visit groups

413   of resources that are available at different times during the day. Evidence supporting this

414   comes from the fact that, the temporal availability and duration of a resource, such as nectar

415   or pollen, varies from flower to flower (Kleber, 1935; Linnaeus, 1755; Parker, 1926; von Buttel-

416   Reepen, 1903). In addition, bees foraging to a food source that is available at noon or late in

417   the afternoon have been shown to scout the food source on average up to 4 hours, prior to

418   the resource availability on earlier days (Moore & Doherty, 2009; Moore & Rankin, 1993;

419   Moore et al., 1989). Furthermore, once the resource a forager is exploiting closes for the day,

420   the forager goes into the hive and does not take additional foraging flights for the day (Körner,

421   1940; Moore et al., 1989; Seeley, 1995; von Buttel-Reepen, 1903; von Frisch, 1940). It is

422   possible that constant workers in our study are foraging to food sources available early in the

423   afternoon, while afternoon shift workers are foraging to food source available in the late

424   evening, but further studies are needed to test this hypothesis.

425   Alternatively, constant foragers could be classified as reticent foragers, who wait in the

426   dance floor for a food source to be announced and forage as recruited by other individuals

427   (Moore et al., 2011; Wagner et al., 2013). Another possibility is that the observed shift work

428   strategy stems from the availability of stable food sources around the colony. In this scenario,

429   foragers could specialize to more efficiently exploit a particular food source at its highest

430   production point of the day, thus encouraging a shift work strategy. In contrast, a habitat where

431   resources are scarce and constantly changing would foster foragers taking foraging trips at all

432   times. Evidence for this notion stems from studies of different honey bee subspecies in Turkey,

433   where *Apis mellifera syriaca,* which originate from an arid habitat with mild winters, presented

434   higher flower fidelity than *A.m. carnica* and *A.m. caucasica,* which inhabit mountain regions

435   with cold winters and short summers (Cakmak et al., 2010). Since the experiments in the

436   previous study were performed in the same location it is likely that flower fidelity has a genetic

437   component and this component may play a role in the shift work strategy that we observe in

438   the current study.

439   Genotyping efforts by Kraus and colleagues (2011) suggested that shift work might be

440   present and strongly affected by patrilineal genotype. Our findings are consistent with their

441   measures, as pollen foragers with shift make up approximately 8% of the observed pollen

442   specialists (Figure 2A). In contrast, approximately 36% of non-pollen foragers observed

443   presented either a morning or afternoon shift (Figure 2B). This difference in the proportion of

444   shift workers could be the result of intrinsic factors that differentiate pollen and non-pollen

445   foragers, environmental factors such as resource availability or a combination of both. Studies

446   examining resource specialization in foragers demonstrate intrinsic differences between

447   pollen and nectar foragers, such as genetic background, sucrose responsiveness, phototaxis

448   and octopamine titters (Barron et al., 2007; Erber et al., 2006; Giray et al., 2007; Page & Erber,

449   2002; Scheiner et al., 2003, 2001, 2002, 2014; Taylor et al., 1992; Wagener-Hulme et al.,

450   1999). Given the similarity of proportion of shift work in this and the Kraus et. al study, it is

451   likely that shift work in foraging may be dependent on foraging specialization. Alternatively,

452   since pollen and nectar availability varies throughout the day from one flower to another (R.M.

453   Goodwin, 1986; Linnaeus, 1755; Nakamura & Seeley, 2006; Stone, Willmer, & Alexandra

454   Rowe, 1998), it is possible that the difference between pollen and non-pollen foragers stems

455   from the availability of the particular resource a forager exploits. Future studies will examine

456   how resource availability affects foraging timing and strategies and will explore if differences

457   in patrilineal origin of non-pollen foragers influences their foraging shift.

458   We found that a group of individuals may begin foraging in either the morning or

459   afternoon shift and over time switch shifts (Figure 3A). This switch is more probable to occur

460   early in the foraging life (Figure 3C). This mechanism may be linked to epigenetic, hormonal,

461   developmental or morphological changes occurring after the onset of foraging behavior

462   (Brown, Napper, & Mercer, 2004; Farris, Robinson, & Fahrbach, 2001; Withers, Fahrbach, &

463   Robinson, 1995). Since honeybee colonies need to constantly adapt to changes in the outside

464   environment and resource availability, having a foraging force that can adjust at a moment's

465   notice may result in a constant flow of resources into the colony. Alternatively, it is possible

466   that changes in the timing of foraging result from the disappearance of the resource the bee

467   was exploiting, causing her to visit a new resource that may be available at a different time.

468   Although much work remains to be done, both of these scenarios are consistent with the idea

469   that shift work may be plastic and thus adopts to the colony's constant needs.

470   While our findings show that some individuals perform foraging in shifts, our direct

471   observations of foraging behavior cannot determine if shifts are intrinsic or a function of

472   external factors. While assaying foraging we also observed fanning at the entrance of the

473   colony. To our surprise, we found that some individuals fanned exclusively in the morning or

474   afternoon, while others had no preference for a specific shift (Figure 4). The observed shifts

475   in fanning suggest that shift work may have one or more intrinsic drivers. One of these drivers

476   may be genetic variation among individuals in the colony, as previously described for pollen

20

477   foragers (Kraus et al., 2011). Previous studies looking at genetic variation within fanning bees

478   found that colonies with natural genetic variation have a more rigorous control of temperature

479   inside of the colony (via fanning) in comparison with colonies that originate from a single

480   artificially inseminated queen (J C Jones et al., 2004; Julia C Jones, Nanork, & Oldroyd, 2007;

481   Su et al., 2007). Furthermore, evidence suggests that genetic variation in the colony increases

482   overall colony fitness (Mattila & Seeley, 2007). Taken together, our data on fanning task and

483   that of previous studies, it is possible that shift work in honeybees has one or more intrinsic

484   mechanisms driving it. If this driver or drivers have a genetic component, the study of single-

485   cohort colonies may result in the loss of one or both shifts in foraging and fanning tasks.

486   Since some of the marked individuals we observed foraging also fanned, we explored

487   the potential relationship of shift work between these tasks. Our results revealed that while a

488   proportion of individuals (30%) perform foraging and fanning behaviors at different time

489   periods, the remaining individuals presented the same shift for both foraging and fanning

490   (Figure 5). This suggests that while these tasks may share a relationship with regards to shifts,

491   they can be dissociated from one another (Figure 5B). This difference between foraging and

492   fanning shifts could be explained by differences in the influences of endogenous (genetic

493   background, life stage) and exogenous factors (light, temperature, resource availability, colony

494   needs). Previous work done using the fruit fly *Drosophila melanogaster*, uncovered

495   experimental proof of the multiple circadian oscillator hypothesis originally proposed by Dan

496   and Pittendrigh (Colin S. Pittendrigh & Daan, 1976; Stoleru, Peng, Agosto-Rivera, & Rosbash,

497   2004). This hypothesis states that complex multicellular organisms possess various

498   independent or loosely coupled circadian pacemakers (C. S. Pittendrigh, 1972). In the case

499   of the fruit fly researchers uncovered that different cells were responsible for the morning and

500   evening activity peaks in locomotor behavior (Stoleru et al., 2004). Similarly, we hypothesize

501   that each task (foraging and fanning) is under a set of different circadian oscillators and while

502   the oscillators may be in synchrony in some individuals, this may vary across individuals.

503   Based on our observation of a shift work strategy in foraging and fanning tasks, we

504   posit that the use of this strategy may confer various benefits to honeybee colonies. The use

505   of shift work in foraging will allow the colony to take advantage of stable resources available

506   throughout the day. Constant workers could enhance the efficiency of shift workers by being

507   ready to forage when a food source is announced, thus increasing the number of foraging

508   flights to the particular food source. Although having shift work can provide a number of

509   benefits to the hive, it may have negative effects on the individual. For example, if we presume

510   that phase differences in the circadian clock underlie the observed shift work, then some

511   individuals may be desynchronized with respect to environmental cycles. A number of studies

512   in humans have shown that individuals with evening chronotypes have increased susceptibility

513   to a number of disorders such as circadian misalignment, cancer and depression (Adan et al.,

514   2012; Antunes, Levandovski, Dantas, Caumo, & Hidalgo, 2010; Davis & Mirick, 2006; Dibner,

515   Schibler, & Albrecht, 2010; Lépine & Briley, 2011; Reinberg, Touitou, Lewy, & Mechkouri,

516   2010). In the case of honeybees, shift work could potentially have negative effects on the

517   individual workers. Future studies will look at dissecting the relationship between shift work in

518   foraging and fanning behavior and circadian rhythms in bees (Giannoni-Guzmán et al., 2014).

519   In conclusion, this study shows for the first time direct behavioral evidence of shift work

520   strategy being used in foraging and fanning tasks in honeybee colonies and characterize

521   behavioral components of this shift work strategy. These findings reveal yet a new layer of

522   social and temporal organization of honeybee colonies. Future studies may aim to understand

523   the specific genetic components and neural mechanisms underlying shift work. Since

524   honeybees use their endogenous circadian clock to predict time of day, the relationship

525   between circadian rhythms and shift work is an area of great interest. Studying this relationship

526   may eventually provide clues on how to attack the negative consequences of imposed shift

527   work in humans.

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Acknowledgements:

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