1

Do bumble bee queens choose nest sites to maximize foraging rate?

-Testing models of nest site selection-

Yukari Suzuki, Lina G. Kawaguchi, Dulee T. Munidasa, and Yukihiko Toquenaga

Division of Integrative Environmental Sciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, JAPAN

Complete Affiliation:
Division of Integrative Environmental Sciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Ten-Nou-Dai, Tsukuba City, Ibaraki 305-8572, JAPAN

Corresponding Author:
Yukari Suzuki
Tel.:
+ 81-22-795-6689
Fax:
+ 81-22-795-6689
E-mail:
yukari@m.tains.tohoku.ac.jp
Present Affiliation of Corresponding Author:
Division of Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Sendai 980-8578, Japan

27 ## Abstract

28 We proposed "Foundress-Max" hypothesis that a bumble bee foundress chooses her nest site to

29 maximize her energy intake rate from nectar. To examine the hypothesis, we estimated the

30 maximum energy intake rate at each site in the study area, and compared the distribution of the

31 maximum energy intake rates with those of actual nest sites. We also calculated rank correlations of

32 the maximum energy intake rate with the number of nest-searching foundresses at 54 sites. The nest

33 locations supported the Foundress-Max hypothesis, but the number of nest-searching foundresses

34 did not. This could be attributed to the density of food sites: many food sites may attract many

35 foundresses. Therefore, we subsequently proposed "Foundress-Sum" hypothesis that a foundress

36 chooses her nest site to maximize the sum of energy intake rates. The nest locations supported the

37 Foundress-Max hypothesis more than the Foundress-Sum hypothesis. A profitable food site would

38 affect foundresses' nest site selection.

39

40 Keywords: *Bombus ardens*, net rate of energy intake, nesting site, nest-searching behavior

41

Introduction

Nest location in a given spatial distribution of food resources is an essential factor determining the foraging efficiency in social insects (Dukas and Edelstein-Keshet 1998; Cresswell et al. 2000). In most social insects, a colony occupies a single and immobile nest, and nest mates must go back to their nest after foraging. If the nest is far from food resources, both long traveling times and large energy costs reduce foraging efficiency.

Low foraging efficiency will lead to food shortage in nests, which limits their colony development and reproductive success in social insects. In bumble bees, food shortage causes lower blood temperature and torpor in workers (Heinrich 1979), longer development time of immature bees (Plowright and Pendrel 1977; Sutcliffe and Plowright 1988, 1990; Cartar and Dill 1991), and smaller adults (Sutcliffe and Plowright 1990). Colony persistence tended to be low in the meadows where plants that are highly preferred by *Bombus* were decreased by drought in late season (Bowers 1985).

Since decreases in foraging efficiency will limit colony development and persistence, the locations of bumble bee nests should be chosen to maximize foraging efficiency in a given distribution of food resources. Determinants of bumble bee nest locations include foraging efficiency, landscape (e.g., forest boundary or banks), microclimate (i.e., temperature, light, and humidity), and the availability of nest materials. Among these, we considered foraging efficiency to be the most important because it can affect colony development and colony persistence in bumble bees. In our previous study (Suzuki et al. 2007), we proposed a hypothesis that the nest sites are

61 chosen so as to maximize the sum of foraging efficiency of workers (Worker-Sum hypothesis). We

62 adopted the net rate of energy intake (Dukas and Edelstein-Keshet 1998; Cresswell et al. 2000) as

63 the foraging efficiency, and constructed a model for calculating the sum of net rates of energy

64 intake at each candidate nest site. Our model indicated that bumble bee nests tended to be located at

65 sites where the sum of energy intake rates in mid season was high. It could be attributed to high

66 colony persistence, and queen's tendency to revisit maternal nest. However, its tendency was weak.

67 Hence, we considered that the foraging efficiency of queens who forage before colony

68 establishment (hereafter foundresses) would be more important than worker's foraging

69 efficiency. Since foundresses choose their nest sites, the net rate of energy intake of a foraging

70 foundress is most likely to affect their nest site selection.

71 Here, we proposed a new hypothesis named the Foundress-Max hypothesis that the nest

72 site is chosen to maximize the net rate of energy intake of a foundress before colony establishment.

73 Based on the Foundress-Max hypothesis, we constructed a new model to calculate the maximum

74 value of the net rates of energy intake. We estimated the maximum energy intake rate at each

75 candidate nest site and found feral nests of *Bombus ardens* Smith in the study area. To examine this

76 hypothesis, we compared the distribution of the maximum energy intake rates with those of the

77 actual nest sites. In addition, we calculated rank correlations between the maximum energy intake

78 rate and variables of foundresses' nest-searching behaviors at 54 sites. We discuss the effect of

79 foraging efficiency on foundress nest site selection, and the application of our model to finding the

80 nest locations of bumble bees and other social insects.

81

Model

NET RATE OF ENERGY INTAKE

84 We used the net rate of energy intake (Dukas and Edelstein-Keshet 1998; Cresswell et al. 2000) as a

85 foraging efficiency. The net rate of energy intake ψ (J/s) is expressed as follows:

86
$$\psi = \frac{(\text{energy from nectar}) - (\text{flight cost})}{(\text{foraging time}) + (\text{flight time})} \qquad (1)$$

87 We set sugar intake from nectar to be a determinant of energy intake rate. Pollen is also an

88 important floral resource for queens and larvae. It would be ideal to incorporate the net energy

89 intake rate of pollen into our model, but this is difficult to estimate because foragers can get energy

90 from nectar when they collect pollen. The importance of pollen is protein intake that will influence

91 colony success after colony establishment.

92   For calculation of the net rate of energy intake ψ based on field data, we neglected a

93 cost of flying during foraging: the cost of flying between flowers within a flower patch and the cost

94 of flying between flower patches within a food site. In addition, we assumed a simple foraging

95 process — a forager goes straight to a food site, fills its honey stomach at the food site, and returns

96 to the nest (Suzuki et al. 2007). Thanks to this simplification, the calculation of net energy intake

97 rate can be calculated from measurable and relatively few field data (Table 1). The field data were

98 measured in this study (see Materials and Methods).

99   The area was divided into square cells, and the locations of the food and nest sites were

100 defined according to these cells as (i, j) and (x, y), respectively. The energy from nectar is calculated

101 as the product of C (μl), S_{ij} (mg/μl), and E (J/mg) (Table 1). C is the volume of honey

102 stomach. S_{ij} corresponds to the sugar concentration of nectar at the food site (i, j). S_{ij} can be

103 measured in the field. E is the energy gain from sugar.

104 $$\text{energy from nectar} = CS_{ij}E \tag{2}$$

105 The cost of flying is calculated from M (J/mg/s), W_{ij} (mg), and the flight time. M is a cost of

106 flying per mg of bumble bee's body weight per second. W_{ij} represents the body weight of a

107 foraging bumble bee at the food site (i, j). The flight time is given by D (m) divided by V (m/s).

108 D is twice the Euclidean distance between the food site (i, j) and the nest site (x, y).

109 $$\text{flight cost} = \frac{2W_{ij}M\sqrt{(i-x)^2 + (j-y)^2}}{V} \tag{3}$$

110 W_{ij} represents the body weight of a bumble bee with its honey stomach half filled, which

111 approximates the average body weight during the foraging process. According to Comba et al.

112 (1999), the weight of one unit of sucrose solution $Ws(N)$ (mg/μl), where N is sucrose weight

113 per 100 g of solution, can be calculated by

114 $$Ws(N) = 0.0000178N^2 + 0.0037821N + 0.9988603. \tag{4}$$

115 We set the body weight of a bumble bee with an empty honey stomach at 200 mg that is an average

116 weight of bumble bees, and the weight of $0.5C$ μl of sucrose solution was calculated as

117 $0.5CWs(N)$. Hence, the body weight becomes $W_{ij} = 200 + 0.5CWs(N_{ij})$. The foraging time is

118 considered as the time to fill the honey stomach, or $(CH_{ij})/R_{ij}$. H_{ij} and R_{ij} can be measured in

119 the field. The sum of the foraging time and the flight time becomes

120 $$\text{foraging time} + \text{flight time} = \frac{CH_{ij}}{R_{ij}} + \frac{2\sqrt{(i-x)^2 + (j-y)^2}}{V} \qquad (5)$$

121   From Eqs. (1), (2), (3), (4) and (5), the net rate of energy intake of a forager that flies to

122 the food site (i, j) from the nest site (x, y) is given by

123 $$\psi(x,y,i,j) = \frac{CS_{ij}E - \dfrac{2W_{ij}M\sqrt{(i-x)^2 + (j-y)^2}}{V}}{\dfrac{CH_{ij}}{R_{ij}} + \dfrac{2\sqrt{(i-x)^2 + (j-y)^2}}{V}} \qquad (6)$$

124 The set of ψ at a candidate nest site (x,y) becomes $\Psi_{x,y} = \{\psi(x,y,0,0), \psi(x,y,1,0), \ldots\}$.

125

126 ## MODEL BASED ON THE FOUNDRESS-MAX HYPOTHESIS

127 In spring, bumble bee foundresses wake up from their hibernation and start searching for suitable

128 nest sites. Since a foundress must build her nest and feed her larvae by herself before the emergence

129 of worker adults, she should choose a suitable nest site near a food site where she gets a better net

130 rate of energy intake. Hence, we proposed the Foundress-Max hypothesis that a nest site is chosen

131 to maximize the net rate of energy intake of a foraging foundress before colony establishment.

132   In adopting the Foundress-Max hypothesis, we assumed that a given candidate nest site

133 (x,y) is evaluated according to the maximum value of a set of net rates of energy intake from the

134 candidate nest site, denoted by $\mu(x,y)$, in the nest-building season.

135 $$\mu(x,y) = \max \ \Psi_{x,y} \qquad (7a)$$

136 $$\Psi_{x,y} = \{\psi(x,y,i,j)\}_{i=0, j=0}^{\max x, \max y} \qquad (7b)$$

137 where $\Psi_{x,y}$ is the set of the net rate of energy intake ψ from the candidate nest site (x, y) to each

138   food site (i, j). We hypothesized that the probability of colony establishment at (x, y) increases as

139   the maximum energy intake rate $(\mu(x,y))$ increases. We developed a computer program in C^{++} and

140   ran it on a Linux operating system.

141

Material and Methods

143   BUMBLE BEES

144   *Bombus ardens* Smith is commonly observed in meadows, suburbs, and towns in Japan. At the end

145   of March, foundresses wake up from their hibernation and search for nest sites. Nests are often

146   established inside the abandoned burrows of rodents, in cavities between rocks and soil, or in gaps

147   between artificial structures. From mid April to early June, workers emerge and forage for floral

148   resources. If the colony grows sufficiently, it produces gynes and males from mid to late June, and

149   then the colony collapses gradually until the end of June.

150

151   STUDY AREA

152   We collected field data in Kasama, Ibaraki Prefecture Japan (N 36° 22', E 140° 15'). The landscape

153   consists of residential areas, parks, paddy fields, woodland, and a mountain. The study area was set

154   to an area of $2.5\,\text{km} \times 2.5\,\text{km}$. We recorded locations where *B. ardens* queens were foraging as

155   "food sites" on a map. Spatial scale of a food site varied from 1 plant (e.g. a tree of *Rhododendron*

156   sp.) to a patch of dozens of plants (e.g. a bush of *Rubus palmatus*). If patches of different plant

157   species were spatially overlapped (though such cases were rare), and both were visited by *B. ardens*,

158   we recorded both species on the map. We divided the study area into 100×100 cells, and

159   projected the points of food sites for each cell ($25\,\text{m} \times 25\,\text{m}$).

160

161   ESTIMATION OF NET ENERGY INTAKE RATE

162   We estimated standing crop R_{ij}, sugar concentration of nectar S_{ij}, and foraging time per flower

163   H_{ij} used in the equation of the net rate of energy intake from field data. The field study of these

164   R_{ij}, S_{ij}, and H_{ij} was conducted from 8th – 29th April 2005. In our study area, different plant

165   species flowered one after another in April. If foundresses made decision on their nest-site selection

166   for a short time, the accuracy of model estimation would be affected by the seasonal change of the

167   flowering plants. To track the seasonal change of the flowering plants, we divided one month into

168   three periods, and measured standing crop (R) and sugar concentration (S) of nectar, and foraging

169   time per flower (H) for each flower species in each period. At food sites where *B. ardens* was

170   foraging, nectar was drawn from flowers with glass microcapillaries (Drummond Microcaps®,

171   Drummond Scientific Co., Broomall, PA, capacity: $0.2, 0.5, 1, 2, 3, 5, 10\ \mu$l), and its volume

172   ($R\ \mu$l) was calculated from the length of the fluid column. Then, its sugar concentration of drawn

173   nectar was measured as N (g per 100 g of sucrose solution) with a handheld refractometer

174   (Eclipse; Bellingham and Stanley Ltd., Tunbridge Wells, UK). N (g per 100 g) was converted to

175   S (mg per $1\ \mu$l). We also measured the foraging time of *B. ardens* over 5 to 48 consecutive flower

176   visits. Foraging time per flower (H) was averaged for observations of 1–5 foundresses (8 to 122

177   total flower visits). We excluded the effect of pollen from our evaluation by investigating bumble

178   bee foragers that carry no pollen. We obtained H (sec) as the ratio of the measured foraging time

179   divided by the number of flowers visited. Standing crop of nectar (R) and its sugar concentration

180   (S) were averaged for 5 to 17 flowers of each plant species. We obtained the parameter sets (R, S

181   and H) for five plant species in total. Using R_{ij}, S_{ij}, and H_{ij} measured in the field, we

182   calculated the net rate of energy intake for each period in the study area.

183

184   TESTS OF HYPOTHESES

185   *Comparison of the distribution of the maximum energy intake rate ($\mu(x,y)$) with those at four nest*

186   *sites*

187   The nest search was independent of model prediction. We searched the study area for nests, and

188   found bumble bees by eyes during the nest search in April and May. We usually did not follow the

189   foundresses from the food sites to exclude a sampling bias. We tried to find all nests in the study

190   area, but it is possible that we failed to find some nests because finding feral nests was difficult. We

191   found six natural *B. ardens* nests (colonies I-VI) in the study area in April. Colony I was located at

192   site A, and colonies II and III were located in the same cell, at site B. Colonies IV and V were

193   located at site C, and colony VI was located at site D (Fig. 1).

194   We compared the distribution of the maximum energy intake rate ($\mu(x,y)$) with those of

195   the actual nest sites. We assumed that actual nest sites supported the hypothesis if $\mu(x,y)$ at actual

196   nest sites were included within the upper 5% of the $\mu(x,y)$ distribution. Since the rank of $\mu(x,y)$

197   did not include the number of the cells with the same $\mu(x,y)$, we defined a "hot zone area" as the

198   number of cells with $\mu(x,y)$ that was equal to or higher than $\mu(x,y)$ of the cell for the nest site.

199   Then, the hot zone area of the nest site was less than 500 (i.e. 5% of all cells) if $\mu(x,y)$ at the nest

200   site were included within the upper 5% of the $\mu(x,y)$ distribution. We calculated the hot zone area

201   of each actual nest site using the net rate of energy intake of a foraging foundress in early, mid, and

202   late April.

203

204   *Rank correlation between the average of the maximum energy intake rates and foundress*

205   *nest-searching behaviors*

206   Although we searched a large area for nests, our nest-searching efforts might be biased toward the

207   area close to food sites or where nests of *B. ardens* were previously found. To exclude possible

208   biases of sampling efforts, we investigated nest-searching behavior of foundresses in quadrats

209   selected almost randomly.

210   We selected 54 cells, and set a quadrat as a square area (25×25 m) on the location of each

211   selected cell in the study area. Then, we made a preliminary examination of whether we could

212   conduct our census in the candidate cells in the study area. If we could not conduct our census in

213   the candidate cells due to its geographical feature (e.g. steep slope) or landownership, we set an

214   alternative quadrat nearby. About 10% of the study area is unsuitable to follow nest-searching

215   foundresses due to its geographical feature though we could search for food sites in the area. About

216   30% of the study area is residential area.

217   Observation of foundresses was carried out on 6, 9, 13, 14, and 17 April 2006. Observation

218   time was 9:00 - 10:30 a.m. (first period) and 11:00 a.m. - 12:30 p.m. (second period). Each observer

219   randomly walked searching for bumble bee foundresses about for 90 minutes in the quadrat. Once

220   detected, a foundress was observed continuously until it flew away from the quadrat. We measured

221   (i) the total number of observed foundresses. Observed foundresses were searching for suitable nest

222   sites (flying in a zigzag pattern close to the ground and occasionally landing on and investigating

223   the ground surface), resting on the ground or vegetation, just passing through, or foraging in the

224   quadrat. To exclude the possible correlation between the number of foraging foundresses and the

225   availability of food resources, we also measured (ii) the number of nest-searching foundresses. To

226   evaluate a queen's eagerness to search for nest site in focal quadrat, we also measured (iii) the time

227   spent on the nest-searching behavior.

228   We calculated the rank correlation between the average of the maximum energy intake

229   rates in early and mid April ($\overline{\mu(x,y)}$) and each variable of the foundress nest-searching behaviors.

230   Kendall's tau with blocking variables (Korn 1984) was calculated for each combination, and

231   conditional independence was tested. In this study, observation period (first/second) was treated as

232   a blocking variable.

233

Results 1: Tests of the Foundress-Max hypothesis

COMPARISON OF THE DISTRIBUTION OF THE MAXIMUM ENERGY INTAKE RATE ($\mu(x,y)$)

WITH ACTUAL NEST LOCATIONS

237 The Foundress-Max hypothesis was supported by the actual nest locations. The maximum energy

238 intake rate ($\mu(x,y)$) predicted all actual nest sites using the net rate of energy intake of a foraging

239 foundress in early April (Fig. 1). The hot zone areas of four actual nest sites in early April were less

240 than 500, or 5% of all cells (Table 2). Except for site D, the hot zone areas of the actual nest sites in

241 mid April were also less than 5% of all cells. The hot zone areas of the actual nest sites where there

242 were two colonies (sites B and C) were smaller than those where there was one colony (sites A and

243 D).

244   We also calculated the hot zone areas for the actual nest locations based on the

245 Worker-Sum hypothesis that we proposed in our previous study (Suzuki et al. 2007). The

246 Worker-Sum hypothesis was not supported by the actual nest locations in this study (Table A1 in

247 Appendix A).

248

249 RANK CORRELATION BETWEEN THE AVERAGE OF THE MAXIMUM ENERGY INTAKE RATES

250 ($\overline{\mu(x,y)}$) AND FOUNDRESS NEST-SEARCHING BEHAVIORS

251 79 out of 132 observed foundresses showed nest-searching behavior, whereas only 13 foundresses

252 were foraging in the quadrats. Although the average of the maximum energy intake rates ($\overline{\mu(x,y)}$)

253 was positively correlated with the number of nest-searching foundresses, the Foundress-Max

254 hypothesis was not supported by the foundress nest-searching behaviors. When observation period

255 was treated as a blocking variable, the positive rank correlations between $\overline{\mu(x,y)}$ and the three

256 variables of the foundress nest-searching behavior were not significant (the total number of

257 foundresses: blocked $\tau = 0.1610, P = 0.09566$; the number of nest-searching foundresses: blocked

258 $\tau = 0.1479, P = 0.1258$; time spent on nest searching behavior: blocked $\tau = 0.08601, P =$

259 0.3734).

260

261 ## Alternative hypothesis

262 If the Foundress-Max hypothesis were plausible, the average of the maximum energy intake rates

263 ($\overline{\mu(x,y)}$) and nest-searching behaviors would significantly correlate. However, rank correlation

264 with nest-searching behaviors did not support the Foundress-Max hypothesis. We considered that

265 this might be attributed to the density of food sites. When the density of food sites becomes high,

266 the search areas of various foundresses overlap (Fig. 2). As the number of nest-searching

267 foundresses increases, the probability of colony establishment also increases if the availability of

268 nest sites is not limiting.

269 Hence, we considered an alternative hypothesis: the Foundress-Sum hypothesis that a nest

270 site may be chosen to maximize the sum of the net rates of energy intake of a foraging foundress

271 before colony establishment. In adopting the Foundress-Sum hypothesis, we assumed that a given

272 candidate nest site (x,y) is evaluated according to the sum of net rates of energy intake of a

273 foraging foundress from the candidate nest site, denoted by $\sigma(x,y)$, before the nest-building

274 season.

275 $$\sigma(x,y) = \sum_{i}^{max.x} \sum_{j}^{max.y} \psi(x,y,i,j) \tag{8}$$

14

276   We had hypothesized that the probability of colony establishment at (x, y) increases as the sum of

277   energy intake rates ($\sigma(x,y)$) increases. We calculated the hot zone area based on the

278   Foundress-Sum hypothesis using the net rate of energy intake of a foraging foundress in early, mid,

279   and late April. We also calculated the rank correlations between the average of the sum of energy

280   intake rates in early and mid April ($\overline{\sigma(x,y)}$) based on the Foundress-Sum hypothesis and each

281   variable of foundress nest-searching behaviors in the 54 quadrats.

282

283 ## Results 2: Tests of the Foundress-Sum hypothesis

284 COMPARISON OF THE DISTRIBUTION OF THE SUM OF ENERGY INTAKE RATES ($\sigma(x,y)$)

285 WITH ACTUAL NEST LOCATIONS

286   The Foundress-Sum hypothesis was partially supported by the actual nest locations. Hot zone areas

287   for two actual nest sites were less than 5% of all cells in the case of using the net rate of energy

288   intake of a foraging foundress in early and mid April (Table 3). Although the Foundress-Max

289   hypothesis was supported by more actual nest sites than the Foundress-Sum hypothesis, the hot

290   zone areas based on the Foundress-Sum hypothesis were smaller than those based on the

291   Foundress-Max hypothesis at site B in early April, and at site C in mid April (Tables 2 and 3).

292

293 RANK CORRELATION BETWEEN THE AVERAGE OF THE SUM OF ENERGY INTAKE RATES

294 ($\overline{\sigma(x,y)}$) AND FOUNDRESS NEST-SEARCHING BEHAVIORS

295   The Foundress-Sum hypothesis was supported by the foundress nest-searching behaviors. The

296 positive rank correlation between the average of the sums of energy intake rates based on the

297 Foundress-Sum hypothesis ($\overline{\sigma(x,y)}$) and the total number of foundresses was significant (blocked

298 $\tau = 0.1951$, $P = 0.004348$; the number of nest-searching foundresses: blocked $\tau = 0.1718$, $P =$

299 0.07548; time spent on nest searching behavior: blocked $\tau = 0.1218$, $P = 0.2077$). When the data

300 collected at different observation periods were analyzed separately, the positive correlation between

301 $\overline{\sigma(x,y)}$ and the total number of foundresses, and the positive correlation between $\overline{\sigma(x,y)}$ and the

302 number of nest-searching foundresses in the first period also became significant (Fig. 3).

303

304 ## Discussion

305 Here we proposed two hypotheses: Foundress-Max and Foundress-Sum. In the Foundress-Max

306 hypothesis, nest site is chosen to maximize the net rate of energy intake of a foraging foundress

307 before colony establishment. In the Foundress-Sum hypothesis, nest site is chosen to maximize the

308 sum of net rates of energy intakes of a foraging foundress before colony establishment. Through

309 field investigation, we estimated the maximum energy intake rate ($\mu(x,y)$) and the sum of energy

310 intake rates ($\sigma(x,y)$) at each site in the study area. We found six feral nests at four nest sites, and

311 investigated foundress nest-searching behaviors at 54 quadrats in the study area. To examine the

312 hypotheses, we compared the $\mu(x,y)$ distribution or the $\sigma(x,y)$ distribution with those of the

313 actual nest sites, and the rank correlation with foundress nest-searching behavior.

314   The Foundress-Max hypothesis was supported by the actual nest locations (Fig. 1). The

315 maximum energy intake rates ($\mu(x,y)$) at four actual nest sites were included within upper 5% of

316   the $\mu(x,y)$ distribution in early April, and $\mu(x,y)$ at three actual nest sites were included within

317   upper 5% of the $\mu(x,y)$ distribution in mid April (Table 2). The Foundress-Sum hypothesis was

318   partially supported by the actual nest locations. The sums of energy intake rates ($\sigma(x,y)$) at two

319   actual nest sites were included within upper 5% of the $\sigma(x,y)$ distribution in early and mid April

320   (Table 3). In addition, the number of nest-searching foundresses in the morning was significantly

321   correlated with $\overline{\sigma(x,y)}$ in early and mid April (Fig. 3). This result suggests that foundresses need

322   more energy to maintain their body temperature during the relatively colder morning period. During

323   field observation, foundresses seemed to search areas far from food sites in the afternoon. They

324   sometimes tried to build their nests in the sites far from food resources, but abandoned the

325   incomplete nests after several days (Y. Toquenaga, personal communication). This may be

326   attributed to low net rates of energy intake. On the other hand, the Worker-Sum hypothesis

327   proposed in our previous study (Suzuki et al. 2007) was not supported here (Appendix A and Table

328   A1).

329   Based on these results, we concluded that a bumble bee foundress chooses her nest sites

330   to maximize her net rate of energy intake before colony establishment. However, our results raised

331   two questions: First, what factors contributed to the higher accuracy of the maximum energy intake

332   rate ($\mu(x,y)$) based on the Foundress-Max hypothesis than of the sum of energy intake rates

333   ($\sigma(x,y)$) based on the Foundress-Sum hypothesis in predicting the actual nest locations? The nests

334   were located at sites with higher $\mu(x,y)$, but the number of nest-searching foundresses in the

335   morning was significantly correlated with $\overline{\sigma(x,y)}$. Second, why was the Worker-Sum hypothesis

17

336   not supported in this study? In our previous study (Suzuki et al. 2007), we had proposed the

337   Worker-Sum hypothesis that nest site is located to maximize the sum of net rates of energy intake

338   of a foraging worker after colony establishment. When we calculated the sum of energy intake rates

339    $(I(x,y))$ based on the Worker-Sum hypothesis at the actual nest sites, the sum of $I(x,y)$ at the

340   actual nest sites was significantly higher than that of sites selected randomly in the case of using the

341   net rate of energy intake of a foraging worker in mid May. However, in this study, the sum of

342    $I(x,y)$ at the actual nest sites was not significantly higher than that of sites selected randomly. In

343   the following two subsections, we discuss these two questions. In addition, we propose an

344   application of our model to finding the nests of central-place foragers in the last subsection.

345

346   NEST LOCATIONS AND NEST-SEARCHING FOUNDRESSES

347   The maximum energy intake rate $(\mu(x,y))$ based on the Foundress-Max hypothesis was more

348   accurate for predicting the actual nest locations than the sum of energy intake rates $(\sigma(x,y))$ based

349   on the Foundress-Sum hypothesis. In the Foundress-Max hypothesis, an area with high $\mu(x,y)$

350   means an estimated nest area that does not take nest density into account. In the Foundress-Sum

351   hypothesis, an area with high $\sigma(x,y)$ means an estimated area with high foundress density and

352   high nest density, which results in low $\sigma(x,y)$ with low nest density. Hence, $\sigma(x,y)$ was

353   effective for predicting the density of foundresses whereas $\mu(x,y)$ was effective for predicting nest

354   locations regardless of nest density.

355   In addition, competition among foundresses may have affected the probability of colony

18

356   establishment. A high foundress density may lead to competition for floral resources and nest sites.

357   Since foundresses compete for nest sites and sometimes fight to the death, the probability of colony

358   establishment would not simply be proportional to the number of nest-searching foundresses. This

359   phenomenon may appear in other animals whose foraging patterns are central-place foraging

360   because competition for nest sites has been reported in various species, especially in birds (e.g.,

361   Renton 2004).

362

363   COLONY PERSISTENCE AND REPRODUCTIVE SUCCESS

364   In our previous study (Suzuki et al. 2007), the Worker-Sum hypothesis was weakly supported by

365   actual nest locations. We had proposed two possible reasons why the sum of the net rates of energy

366   intake of a foraging worker affected colony presence. First, foundresses may choose nest sites based

367   on the food availability before colony establishment, but colonies will go extinct at poor sites after

368   colony establishment. If the first reason is correct, $\mu(x,y)$ based on the Foundress-Max hypothesis

369   or $\sigma(x,y)$ based on the Foundress-Sum hypothesis could estimate the nest site selection by

370   foundresses, and $I(x,y)$ based on the Worker-Sum hypothesis could estimate colony persistence.

371   Second, foundresses select nest sites where food availability will increase after colony

372   establishment by revisiting maternal nest sites. If the second reason is correct, $I(x,y)$ based on the

373   Worker-Sum hypothesis could estimate the nest site selection by foundresses.

374   In this study, $\mu(x,y)$ and $\sigma(x,y)$ led to the successful prediction of actual nest sites.

375   This seems to support the first explanation as stated above. However, we did not observe colony

19

376 extinction, and $I(x,y)$ were low at actual nest sites in 2005. Moreover, colony development seems

377 to be independent of $I(x,y)$, because the colony development and the reproductive success of two

378 colonies at the same site were varied (Table B1 in Appendix B). This result may be attributed to the

379 other factors before colony establishment, e.g., length of the foundress' hibernation (Beekman and

380 van Stratum 2000). Since the positive effects of food availability on colony development and

381 persistence have been discussed in many previous studies in bumble bees (Heinrich 1979; Plowright

382 and Pendrel 1977; Bowers 1985; Sutcliffe and Plowright 1988, 1990; Cartar and Dill 1991;

383 Schmid-Hempel and Schmid-Hempel 1998) and other bees (e.g., Williams and Kremen 2007), we

384 must further examine the effect of the net energy intake rate on colony development, colony

385 persistence and reproductive success in future studies.

386

387 APPLICATION TO FINDING NEST LOCATIONS

388 Bumble bee foraging behavior has been studied in many experiments using commercial colonies

389 (e.g., Osborne et al. 1999; Worden and Papaj 2005; Burns and Thomson 2006), but the ecology of

390 wild bumble bees remains largely unclarified due to the difficulty of finding their nests. In this

391 study, the hot zone area also represents the total area that must be searched to find a nest based on

392 the maximum energy intake rate or the sum of energy intake rates. If we could develop a method to

393 estimate bumble bee nest locations based on our model, it would be a useful tool for future studies.

394 Since bumble bees are major pollinators of numerous plants, the estimation of nest locations would

395 be also useful in pollination ecology and plant conservation ecology.

396   Bumble bees' nest locations can be affected by foraging efficiency, landscape,

397   microclimate, and the availability of nest materials. As well as nectar, pollen is an important floral

398   resource for queens and larvae. If a foundress chooses her nest site to maximize her fecundity and

399   larvae's growth rate at the beginning of colony establishment, the protein intake of pollen should

400   also affects nest locations. Landscape affects the nest site selection by bumble bee foundresses

401   (Svensson et al. 2000; Kells and Goulson 2003), and microclimate is frequently correlated with

402   landscape (Kells and Goulson 2003). The availability of nest materials may also be correlated with

403   landscape. Our model can predict the nest locations of *B. ardens* because *B. ardens* is opportunistic

404   species that is not severely restricted by specific nest materials. However, species that build nests

405   underground (e.g., *B. diversus*, *B. hypocrita*) would need specific nest materials.

406   If landscape can be incorporated into our model, the accuracy of prediction may increase

407   for the case of species that need specific nest materials. Lonsdorf et al. (2009) proposed a model of

408   pollinator abundance on a landscape using high-resolution (1m) aerial photographs and GIS.

409   Integration with landscape information using GIS will be necessary for application to finding nest

410   locations. However, we have only a relatively large mesh landscape information ($100m \times 100m$) in

411   the study area whereas cell size in our model was small ($25m \times 25m$). We will have to test whether

412   the landscape information in this scale can represent the spatial heterogeneity of specific nest

413   materials. Further studies are needed to clarify whether our model can be applied to estimate nest

414   locations of the same species in different areas, to estimate nest locations of different bumble bee

415   species, and to estimate nest locations of different species whose foraging pattern is central-place

416   foraging.

417

418   Acknowledgements

424

425   References

426   Allen, T., Cameron, S., McGinley, R., and Heinrich B. (1978) The role of workers and new queens

427   in the ergonomics of a bumblebee colony (Hymenoptera: Apoidea). *Journal of the Kansas*

428   *Entomological Society* **51**, 329-342.

429   Beekman, M. and van Stratum, P. (2000) Does the diapause experience of bumblebee queens

430   *Bombus terrestris* affect colony characteristics? *Ecological Entomology* **25**, 1-6.

431   Bowers, M.A. (1985) Bumble bee colonization, extinction, and reproduction in subalpine meadows

432   in Northeastern Utah. *Ecology* **66**, 914-927.

433   Burns, J.G. and Thomson, J.D. (2006) A test of spatial memory and movement patterns of

434   bumblebees at multiple spatial and temporal scales. *Behavioral Ecology* **17**, 48-55.

435   Cartar, R.V. and Dill, L.M. (1991) Costs of energy shortfall for bumble bee colonies: predation,

436 social parasitism, and brood development. *Canadian Entomologist* **123**, 283-293.

437 Comba, L., Corbet, S.A., Barron, A., Bird, A., Collinge, S., Miyazaki, N. and Powell, M. (1999)

438 Garden flowers: Insect visits and the floral reward of horticulturally-modified variants. *Annals of*

439 *Botany* **83**, 73-86.

440 Cresswell, J.E., Osborne, J.L. and Goulson, D. (2000) An economic model of the limits to foraging

441 range in central place foragers with numerical solutions for bumblebees. *Ecological Entomology* **25**,

442 249-255.

443 Dukas, R. and Edelstein-Keshet, L. (1998) The spatial distribution of colonial food provisioners.

444 *Journal of Theoretical Biology* **190**, 121-134.

445 Ellington, C.P., Machin, K.E. and Casey, T.M. (1990) Oxygen consumption of bumblebees in

446 forward flight. *Nature* **347**, 472-473.

447 Heinrich, B. (1979) *Bumblebee Economics*. Harvard University Press, Cambridge.

448 Kells, A.R. and Goulson, D. (2003) Preferred nesting sites of bumblebee queens (Hymenoptera:

449 Apidae) in agroecosystems in the UK. *Biological Conservation* **109**, 165-174.

450 Korn, E.L. (1984) Kendall-tau with a blocking variable. *Biometrics* **40**, 209-214.

451 Lonsdorf, E., Kremen, C., Ricketts, T., Winfree, R., Williams, N., and Greenleaf, S. (2009)

452 Modelling pollination services across agricultural landscapes. *Annals of Botany* doi:

453 10.1093/aob/mcp069

454 Osborne J.L., Clark, S.J., Morris, R.J., Williams, I.H., Riley, J.R., Smith, A.D., Reynolds, D.R. and

455 Edwards, A.S. (1999) A landscape-scale study of bumble bee foraging range and constancy, using

456   harmonic radar. *Journal of Applied Ecology*. **36**, 519-533.

457   Plowright, R. C. and Pendrel, B. A. (1977) Larval growth in bumble bees (Hymenoptera: Apidae).

458   *Canadian Entomologist* **109**, 967-973.

459   Renton, K. (2004) Agonistic interactions of nesting and nonbreeding Macaws. *The Condor* **106**,

460   354-362.

461   Schmid-Hempel, R. and Schmid-Hempel, P. (1998) Colony performance and immunocompetence

462   of a social insect, *Bombus terrestris*, in poor and variable environments. *Functional Ecology* **12**,

463   22-30.

464   Sutcliffe, G. H. and Plowright, R. C. (1988) The effects of food supply on adult size in the bumble

465   bee *Bombus terricola* Kirby (Hymenoptera: Apidae). *Canadian Entomologist* **120**, 1051-1058.

466   Sutcliffe, G. H. and Plowright, R. C. (1990) The effects of pollen availability on development time

467   in the bumble bee *Bombus terricola* K. (Hymenoptera: Apidae). *Canadian Journal of Zoology* **68**,

468   1120-1123.

469   Suzuki, Y., Kawaguchi, L.G. and Toquenaga, Y. (2007) Estimating nest locations of bumblebee

470   *Bombus ardens* from flower quality and distribution. *Ecological Research* **22**, 220-227.

471   Svensson, B., Lagerlöf, J., and Svensson, B.G. (2000) Habitat preferences of nest-seeking bumble

472   bees (Hymenoptera: Apidae) in an agricultural landscape. *Agriculture, Ecosystems and*

473   *Environment* **77**, 247-255.

474   Williams, N.M. and Kremen, C. (2007) Resource distributions among habitats determine solitary

475   bee offspring production in a mosaic landscape. *Ecological Applications* **17**, 910-921.

476   Worden B.D. and Papaj, D.R. (2005) Flower choice copying in bumblebees. *Biology Letters* **1**,

477   504-507.

78   Appendix A: Worker-Sum hypothesis

79   In our previous study, we had proposed the Worker-Sum hypothesis that nest site is located to maximize

80   the sum of net rates of energy intake of a foraging worker after colony establishment (Suzuki et al.

81   2007). In adopting the Worker-Sum hypothesis, we had assumed that a given candidate nest site (x,y)

82   is evaluated according to the sum of net rates of energy intake of a foraging worker from the candidate

83   nest site, denoted by $I(x,y)$, after the nest-building season. The equation of $I(x,y)$ is the same as Eq.

84   (8) in the Foundress-Sum hypothesis, however, the net rate of energy intake of a foraging worker after

85   colony establishment is used in the equation of $I(x,y)$. We had hypothesized that the probability of

86   colony establishment at (x, y) increases as the evaluation value $I(x,y)$ increases.

87   The Worker-Sum hypothesis was not supported in this study. $I(x,y)$ predicted no actual nest

88   sites in May and June (Table A1). In Suzuki et al. (2007), the sum of $I(x,y)$ at the actual nest sites

89   using the net rate of energy intake of a foraging worker in mid May was significantly higher than that of

90   sites sampled randomly in a randomization test. However, the sum of $I(x,y)$ at actual nest sites was not

91   significantly higher than those of sites sampled randomly in this study (e.g., P=0.334887 in mid May).

92

93   Appendix B: Measurement of colony development

04   We dug out colonies II and III from site B, and colonies IV and V from site C after maturation of

05   sexuals in colonies. The physical size of each nest and the total number of cocoons were measured as the

06   indicators of colony development. Large cocoons were also counted because they can be considered as

07   cocoons of sexuals. Cocoons having a width greater than 10 mm were regarded as large cocoons.

08

Figure 1

(a)
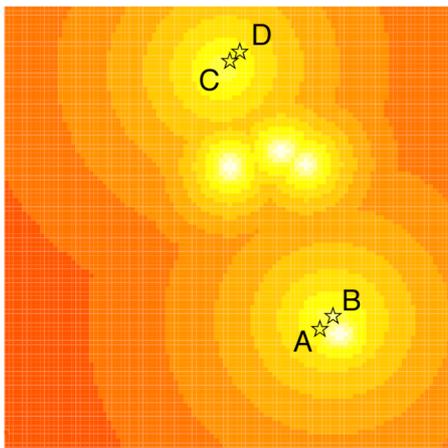
(b)
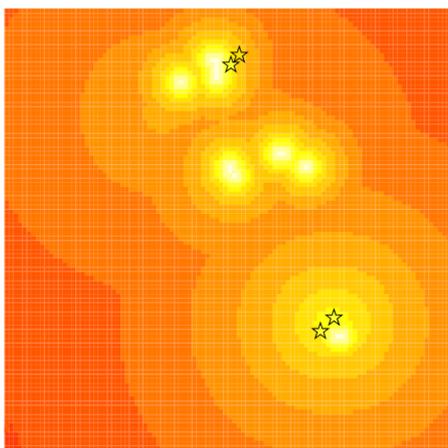
(c)
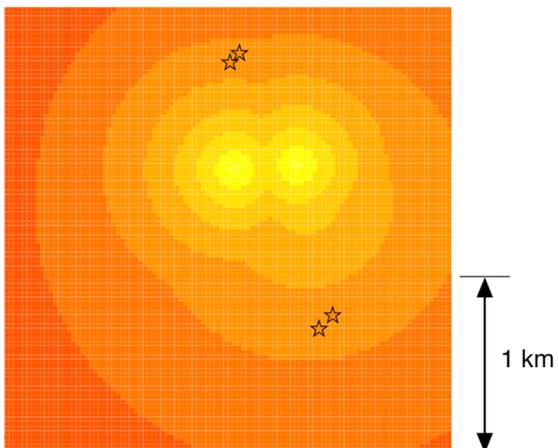
0 ~ 0.21~0.42~0.63~ 0.8 ~1.05~1.26~1.47~1.68~1.89~ 2.1 (J/s)

Figure 2

Low density of food site
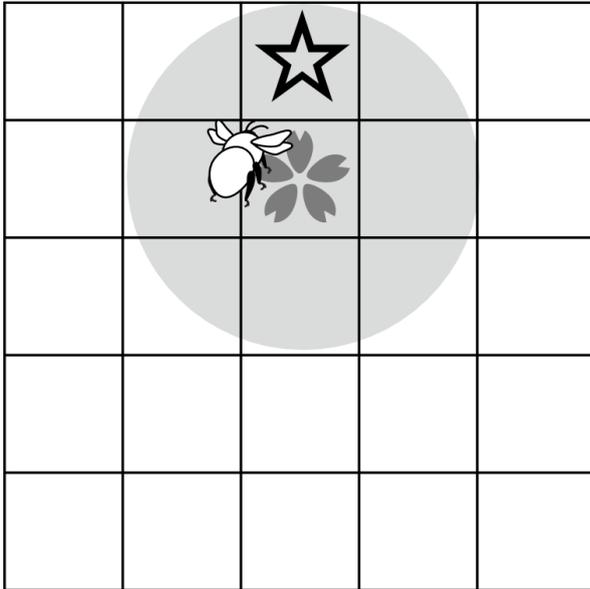
High density of food site
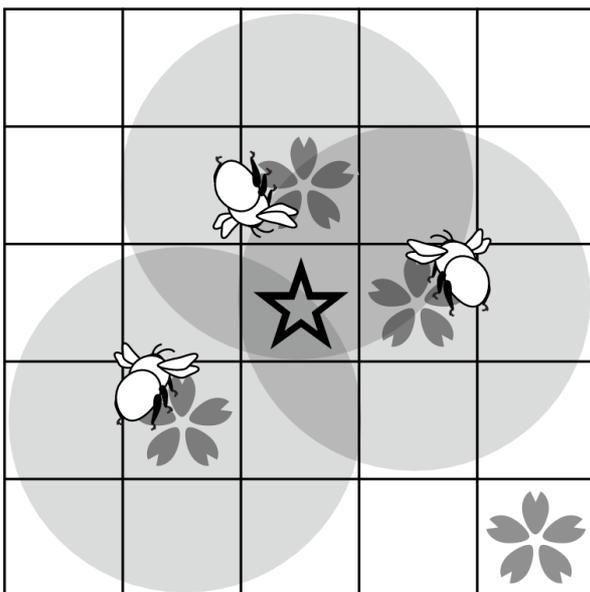
Food site

Foundress

Nest site

Search area

Figure 3

First observation period

Second observation period

No. of observed foundresses

τ b = 0.378
P = 0.0056*

τ b = 0.0118
P = 0.93

No. of nest-searching foundresses

τ b = 0.296
P = 0.030*

τ b = 0.0477
P = 0.73

Time spent on nest searching

τ b = 0.238
P = 0.082

τ b = 0.00572
P = 0.97

Evaluation value based on Foundress-Sum hypothesis