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Original article

Longevity of *Bombus terrestris* workers (Hymenoptera: Apidae) in relation to pollen availability, in the absence of foraging

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Abstract – The need for protein of *Bombus terrestris* workers was investigated by examining the effect of pollen availability on their longevity. Workers were kept under three conditions: provided with sugar solution only, with sugar solution and pollen ad libitum, and with sugar solution and pollen for 6 hours. Worker survival curves differed significantly among all conditions. Pollen deprivation led to a strong decline in survivorship whereas access to pollen for 6 hours only had a small and transient positive effect. This indicates that adult bumblebees have an ongoing need for protein food throughout their lives and confirms the observation that workers that eat pollen without subsequently feeding larvae do so for their own needs. There was no correlation between worker size and longevity, indicating that the reported higher mortality of foragers is not due to their bigger size. Our results show that, when environmental variables are excluded, workers die of senescence after about two months.

***Bombus terrestris* / longevity / pollen availability / protein / social insect**

1. INTRODUCTION

Bees use pollen, which they collect from flowers, as the prime protein resource for raising larvae. In pollen storing bumblebee species, like *Bombus terrestris* L., foragers deposit pollen in pollen storage pots. Smeets and Duchateau (2001) found that *B. terrestris* workers that eat pollen from storage pots for a period longer than about 2 minutes, are very likely subsequently to feed larvae (progressive feeding). On the contrary, workers that eat pollen for a short time (less than about 2 minutes) usually do not feed larvae. The latter has been observed in newly emerged as well as older workers (Duchateau, unpublished data) and supposedly serves to satisfy the workers' own nutritional needs. Bumblebee workers need pollen (protein) for the development of their ovaries (Duchateau, 1989) and for the glandular secretions they add to nectar/sugar water while drinking this (Pereboom, 2000). On the basis of pollen supplement consumption rates of groups of workers, Malone et al. (2000) suggested that bumblebees have an unchanging requirement for protein throughout adult life. However, the effect of pollen deprivation on the longevity of bumblebee workers has not yet been investigated. Bumblebee survivorship curves have been obtained for various species under field conditions (Brian, 1952; Garófalo, 1978; Rodd et al., 1980; Goldblatt and Fell, 1987; Silva-Matos and Garófalo, 2000). Silva-Matos and Garófalo (2000) found that mortality rates in *Bombus atratus* were correlated with the foraging rates of the

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worker. This supports the common idea that worker longevity is affected by environmental hazards associated with foraging, such as predation and bad weather conditions (see e.g. Rodd et al., 1980). Rodd et al. (1980) considered the contribution of senescence to the increase in worker mortality rate to be insignificant. In this study, we investigated the workers' need for protein by comparing the longevity of pollen deprived workers with that of workers fed pollen and sugar solution ad libitum, while excluding the influence of environmental factors. In our study, the survivorship curves of the workers that were fed ad libitum showed the effect of senescence on worker mortality.

Foragers are known to be bigger than house bees (Brian, 1952; Garófalo, 1978) and they have a higher mortality (Brian, 1952; Garófalo, 1978; Katayama, 1996). This higher mortality of foragers has been attributed to the foraging conditions only (Rodd et al., 1980; Goldblatt and Fell, 1987; Katayama, 1996). However, the possibility of an effect of worker size on mortality has never been considered. Therefore, we investigated also whether there was a correlation between worker size and longevity.

2. MATERIALS AND METHODS

Colonies of *Bombus terrestris* were reared under standard laboratory conditions in a climate room (28 °C, 60% RH) and provided with fresh frozen mixed floral pollen, collected by honey bees, and 1:1 sugar solution ad libitum (Duchateau and Velthuis, 1988).

Worker pupae were taken from different colonies and placed randomly in standard nest boxes (internal dimensions 27.5 × 17.5 × 16 cm). Newly emerged workers were marked daily with a small numbered tag. The queenless workers were kept under three conditions: (1) Workers provided with sugar solution only (S only). (2) Workers provided with pollen and sugar solution ad libitum (S + P). (3) Workers allowed to eat pollen for six hours within a day after emergence, after which they were provided with sugar solution only (S + 6 h P). For this, each day's newly emerged workers (n = 2–5) were placed together in a nestbox with male pupae, and provided with sugar solution and fresh pollen ad libitum for 6 hours. After that they were placed back in their home-nestbox, and provided with sugar solution only. A pilot study with video observation of 12 callow workers had shown that all workers ate pollen at least once within six hours. Group (3) was included to investigate the effect of limited protein intake early in adult life. The three groups of workers were also regularly provided with male pupae because providing queenless workers with pupae greatly reduces stress (Duchateau pers. obs.; stressed workers run around a lot and try to escape from their box continuously). The presence of honey pots and eggcups was recorded. The mortality of workers was recorded daily. At the end of the experiment, the size of all workers was determined by measuring the length of the radial cell of their right wing (Medler, 1962).

The S only and the S + P condition were done in duplicate, with an interval of a month between the two repetitions (S only n1 = 14 workers, from 2 different colonies, A and B, n2 = 50 workers, from 5 other colonies, C-G; S + P n1 = 21 from colonies A and B, n2 = 38 workers, from colonies C-G). The S + 6 h P condition was done once, together with the second repetition of the other two conditions (n = 44 workers, from colonies C-G). All worker pupae of the first repetition (n = 14 and n = 21) emerged within 6–7 days, and all worker pupae of the second repetition (n = 50, n = 38 and n = 44) emerged within 9–11 days.

The survivorship curves and medians were determined by the Kaplan-Meier procedure. In this procedure the Breslow test statistic was used for the statistical comparison of the curves.

3. RESULTS

Survivorship curves for the first repetition are shown in Figure 1. The S + P group shows a convex curve, indicating an increasing mortality with age. The S only group shows a similar convex curve with, however, a much sharper decline, indicating that pollen-deprived workers had a higher mortality rate. The median longevity of the two groups in this repetition i.e. the age by which 50% of the workers had died, was 13 and 76 days, for the S only and S + P groups respectively. The worker survival distribution differed significantly between these two groups (Breslow test statistic in Kaplan-Meier procedure = 26.18, df = 1, $P < 0.001$).

Figure 2 shows the survivorship curves for the three groups, S only, S + P and S + 6 h P. The curves of the S only and S + P group are similar to those obtained in the first repetition (S only: Breslow test statistic in Kaplan-Meier

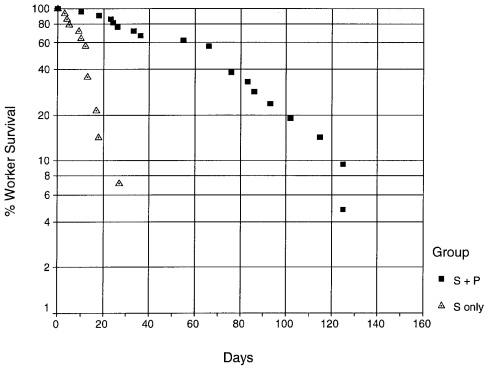

Figure 1. Survivorship curves of two groups of workers, provided with sugar solution only (S only, n = 14) and ad libitum sugar solution and pollen (S + P, n = 21) respectively. Y-axis is logarithmic.

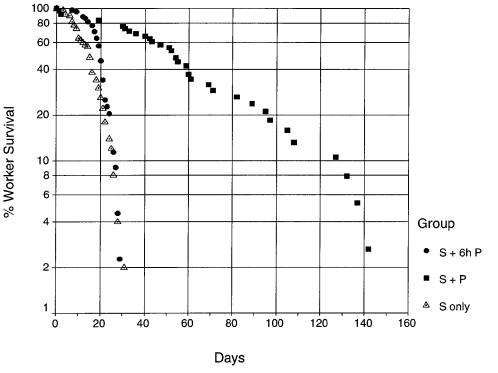

Figure 2. Survivorship curves of three groups of workers, provided with sugar solution only (S only, n = 50), ad libitum sugar solution and pollen (S + P, n = 38) and sugar solution and the opportunity to eat pollen for 6 hours (S + 6 h P, n = 44) respectively. Y-axis is logarithmic.

procedure = 0.63, df = 1, $P = 0.43$; S + P: Breslow statistic = 0.60, df = 1, $P = 0.44$). The curve of the S + 6 h P group shows a sharp decline, similar to that of the S only group, but its onset is delayed. This is reflected in the median longevities, which were 15, 54 and 20 days, for the S only, S + P and S + 6 h P groups respectively. Worker survival distribution differed significantly among the three groups (Breslow test statistic in Kaplan-Meier procedure = 64.10, df = 2, $P < 0.001$, between-group comparison with Bonferroni corrected alfa = 0.017, $P < 0.01$ for all three compared pairs).

The groups of workers provided with both pollen and sugar water constructed most honey pots and were the only group in which eggs were laid: about a week after the start of the experiment the first egg cups were observed. The pollen-deprived workers did not construct as many honey pots as the other two groups and they did not lay eggs.

There was no significant correlation between worker size and longevity in the groups provided with sugar solution and pollen, and the group with limited access to pollen (S + P: total n = 58, r = –0.148, $P = 0.268$; S + 6 h P: n = 44, r = –0.093, $P = 0.549$). In the group provided with sugar solution only, there was a low, but significant, correlation between size and longevity. (S only: total n = 64, r = 0.266, $P = 0.034$).

4. DISCUSSION

The longevity of *B. terrestris* workers in relation to pollen availability was investigated. The overall shape of the survivorship curves of the workers provided with pollen ad libitum was the same as that found in studies of worker mortality under field conditions in temperate climates (Brian, 1952; Rodd et al., 1980; Goldblatt and Fell, 1987; Katayama, 1996). Since in our experimental set-up workers could not forage and only needed to feed a very limited number of larvae (own offspring), our survivorship curves show solely the effect of senescence on the mortality of *B. terrestris* workers. Therefore, it is not surprising that the S + P workers in our experiments survived considerably longer than workers in field colonies (median 76 and 54 days, mean 69 and 61 days), even when compared with house bees of these colonies (mean age of house bees: in *Bombus ardens* 43 days, in *Bombus diversus* 35 days; Katayama, 1996). However, Garófalo (1978) found a mean longevity of 73 days in house bees of the tropical species *Bombus morio*. The difference between house bees and foragers occurs because the latter have a much shorter life span due to the hazards associated with their foraging task (Brian, 1952; Garófalo, 1978; Rodd et al., 1980; Goldblatt and Fell, 1987; Katayama, 1996; Silva-Matos and Garófalo, 2000). The

lack of a correlation between worker size and longevity in our experiments indicates that the higher mortality of foragers found in other studies is, indeed, solely due to the hazards associated with their foraging task (Brian, 1952; Garófalo, 1978; Katayama, 1996), and not to their bigger size.

Both groups of pollen-deprived workers showed high mortality rates compared to workers fed pollen ad libitum. Although workers in the S + 6 h P group lived longer than pollen-deprived workers, the effect of the availability of a limited amount of protein early in adult life was only transient. Therefore, their high mortality must be attributed to their protein shortage, indicating that bumblebees have an ongoing need for protein throughout their lives. This is in accord with Malone et al. (2000), who found that the daily consumption of a pollen supplement by worker bumblebees kept in groups of different sizes did not decline over the lifetime of individual bumblebees. It contrasts, however, with honey bees, where most protein consumption occurs during the first few days of adult life and then drops steadily to very low levels (Crailsheim and Stolberg, 1989). The workers provided with pollen ad libitum not only lived longer, but also constructed egg cups and more honey pots than the pollen deprived workers. This observation supports findings that pollen is instrumental in body maintenance (production of enzymes) (Pereboom, 2000), ovary development (Duchateau, 1989) and wax production.

Taken together, our results support the idea that workers that eat pollen for a short time (less than 2 minutes) in their colony do so to satisfy their own need for protein. Workers eating for a longer period of time do so for the purpose of feeding larvae (Smeets and Duchateau, 2001). About 25% of workers (total n = 148) that were observed eating pollen in a colony did so briefly and subsequently did not feed larvae within 30 minutes after eating (Duchateau, unpublished data). The small amount of pollen eaten on such an occasion suggests that workers eating pollen for their own use do so by consuming a little on a regular basis (about once every 4 hours, Smeets and Duchateau 2001) rather than a large amount every now and then. Malone et al. (2000) found that consumption rates of a pollen supplement (containing 33% floral pollen) by queenless workers of unknown age, ranged from 3.3–35.3 mg/bee/day.

In conclusion, our data show that adult workers of *B. terrestris* have an ongoing need for pollen and that they die of senescence after about 2 months, when they are not exposed to foraging risks, irrespective of their size.

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Résumé – Longévité des ouvrières de *Bombus terrestris* (Hymenoptera, Apidae) en relation avec la disponibilité en pollen, en l'absence de butinage. Chez le bourdon *Bombus terrestris* L. les ouvrières qui consomment du pollen peuvent être divisées grossièrement en deux groupes : (1) celles qui consomment du pollen des pots de stockage pendant plus de deux minutes et vont ensuite nourrir les larves et (2) celles qui au contraire consomment du pollen pendant un laps de temps plus court et ne nourrissent pas les larves. Leur consommation est supposée satisfaire leurs propres besoins nutritionnels protéiques. Le besoin en protéines des ouvrières a été étudié en examinant l'effet de la disponibilité en pollen sur leur longévité. Trois régimes différents ont été offerts à des ouvrières orphelines novices : (1) du sirop seulement (2 répétitions); (2) du sirop et du pollen ad libitum (2 répétitions) et (3) du sirop et la possibilité de consommer du pollen pendant 6 h après l'émergence (une répétition). Le régime 2 montre l'effet de la sénescence sur la mortalité des ouvrières. Les courbes de survie diffèrent significativement selon le régime. La longévité médiane était de 13 et 15 j avec le régime 1 (n1 = 14, n2 = 50), de 76 et 54 j avec le régime 2 (respectivement n1 = 21, n2 = 38) et de 20 j avec le régime 3 (n2 = 44, Fig. 2). La privation de pollen a provoqué un fort déclin de la courbe de survie (Figs. 1 et 2). La possibilité de consommer du pollen pendant 6 j a eu un effet restreint et passager sur la longévité des ouvrières. Ces résultats montrent que les bourdons ont un besoin permanent de nourriture protéique tout au long de leur vie. Il n'y a aucune corrélation entre la taille des ouvrières et leur longévité. Ceci montre que la plus forte mortalité des butineuses trouvée lors des études sur le terrain est due à des problèmes environnementaux et non à leur forte taille. Nos résultats montrent que, si l'on exclut les facteurs du milieu, les ouvrières meurent de vieillesse au bout de deux mois environ, quelle que soit leur taille.

***Bombus terrestris* / longévité / disponibilité en pollen / protéine / insecte social**

Zusammenfassung – Lebensdauer von *Bombus terrestris* Arbeiterinnen (Hymenoptera: Apidae) in Abhängigkeit der Pollenverfügbarkeit ohne Sammeltätigkeit. Pollenverzehrende Arbeiterinnen der Hummel *Bombus terrestris* L. können grob in zwei Gruppen unterteilt werden: die einen fressen länger als 2 Minuten Pollen aus den Vorratstöpfen und füttern daraufhin Larven. Die andere Gruppe frisst kürzer als zwei Minuten und füttert danach gewöhnlich nicht die Larven. Hierdurch befriedigen sie höchstwahrscheinlich ihren eigenen Proteinbedarf. Der Proteinbedarf von Arbeiterinnen wurde anhand des Einflusses der Pollenverfügbarkeit auf ihre Lebensdauer untersucht. Frischgeschlüpfte Arbeiterinnen wurden ohne Königin unter folgenden Bedingungen gehalten: Sie erhielten entweder nur Zuckerlösung, Zuckerlösung und Pollen ad libitum (beide Versuche mit 2 Wiederholungen), oder sie erhielten Zuckerlösung und konnten bis zu 6 Stunden nach dem Schlupf Pollen aufnehmen (1 Wiederholung). Die Versorgung mit Pollen ad libitum zeigt den Einfluss der Alterung auf die Lebensdauer. Die Überlebensdauer unterschied sich signifikant zwischen den Haltungsbedingungen. Die mittlere Lebenserwartung betrug 13 und 15 Tage (nur Zuckerlösung, $n1 = 14$, $n2 = 50$), 76 und 54 Tage (Zuckerlösung und Pollen, $n1 = 21$, $n2 = 38$) und 20 Tage (Zuckerlösung und 6 h Pollenverfügbarkeit, $n2 = 44$; Abb. 2). Der Pollenentzug führte zu einer starken Abnahme in der Überlebenskurve (Abb. 1, 2). Zusammen mit dem kleinen und vorübergehenden Effekt einer sechsstündigen Pollenfütterung zeigt dies, dass adulte Hummeln über ihre gesamte Lebensdauer einen fortgesetzten Bedarf an proteinhaltiger Nahrung haben. Es bestand kein Zusammenhang zwischen der Größe der Arbeiterinnen und ihrer Lebensdauer. Dies zeigt, dass die in Freilandstudien gefundene größere Sterblichkeit auf Umgebungsgefahren, nicht aber auf die Größe der Sammlerinnen zurückzuführen ist. Unsere Ergebnisse zeigen, dass unter Ausschluss von Umgebungsvariablen die Arbeiterinnen unabhängig von ihrer Größe nach etwa 2 Monaten an Altersschwäche starben.

***Bombus terrestris* / Lebensdauer / Pollenverfügbarkeit / Eiweiß / Soziale Insekten**

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