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Effects of sperm on female longevity in the bumble-bee *Bombus terrestris* L.

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The male ejaculate, particularly the accessory gland products, has been shown to affect female survival (as is best understood in *Drosophila melanogaster*). So far, these findings have primarily been discussed in the context of a sexual conflict and multiple mating. Here, we show that in the bumble-bee *Bombus terrestris*, male genotype influences female longevity even though *B. terrestris* generally is a singly mated species and male and female interests may thus be more convergent. In addition, the effect could not be owing to accessory gland products, as we artificially inseminated the queens with the content of the accessory testes only.

Keywords: *Bombus terrestris*; sperm effect; female longevity

1. INTRODUCTION

In many animals, the male ejaculate may have profound and manifold effects on the female, such as an increase in the egg-laying rate and decreased receptivity (e.g. Cordero 1995; Eberhard 1996; Chapman 2001). Several of these effects are caused by proteins in the seminal fluid, which are secreted by the accessory glands (e.g. Chapman 2001; Wolfner 2002). In multiple-mating species, such as *Drosophila*, the effects of the accessory gland products may reduce female longevity (Chapman *et al.* 1995), an observation that has been interpreted in the context of a sexual conflict (Rice 1996; Wolfner 2002). In addition, these effects have been shown to depend on male genotype (Sawby & Hughes 2001). Here, we present a (genotypic) patriline effect on female longevity in the bumble-bee *Bombus terrestris*. The case differs in two fundamental ways from the situation described above. First, *B. terrestris* is mostly singly mated (Schmid-Hempel & Schmid-Hempel 2000), and the male and female should therefore have more common fitness interests. Second, we excluded a possible effect of accessory gland products by injection of the content of the accessory testes only (i.e. sperm and potentially unknown additional substances from the accessory testes).

When the male's genotype affects the life-history parameters of his mate, the possibility for genetic incompatibility between male and female genotypes exists. Genetic incompatibility has been discussed primarily in the context of multiple-mating strategies (Zeh & Zeh 1997), sperm selection or mate choice (Jennions 1997; LeBas 2002), all of which, of course, can also act in singly mated species. In addition, studies have also reported somatic–gametic incompatibility that prevents some sperm from reaching the site of fertilization by the action of macrophages (Bishop *et al.* 1996). Here, we demonstrate that queens receiving sperm from different male lines vary in their survival rate. This suggests a genotypic effect of males on female survival, although the actual mechanism remains unknown.

2. METHODS

Queens of *B. terrestris* were collected in the field in spring 1999 near Zurich, Switzerland. From these, colonies were reared in the laboratory under standard conditions (20–25 °C, 60–70% relative humidity, red light and food *ad libitum*). Virgin sexuals from the second-generation colonies (emerging in spring 2000) were used for this experiment. Males aged 8–15 days were used from five different colonies, thus representing five different patrilines. Queens were taken from eight other colonies, thus representing eight matrilines, and inseminated with sperm from males of the different patrilines at the age of about one week (see Baer & Schmid-Hempel 2000). Each queen was inseminated only once with sperm from one male. Males from different patrilines were used alternately to balance any effect of colony age or insemination practice. For the inseminations, the CO_2-anaesthetized males were dissected and their sperm collected from the accessory testes into a fine glass capillary. The yield in collecting sperm was recorded on an arbitrary scale from 1 to 5 ('sperm collection score': 1, relatively few sperm collected to 5, virtually all sperm collected). We took the sperm collection score as an indication of how readily sperm were available from a given male. In the statistical analyses we treated this score as a factor. However, the conclusions remained the same when it was treated as an ordered factor or as a covariate. One week after insemination, queens were put into artificial hibernation at 4 °C for two weeks. Successful hibernation of artificially inseminated queens is more difficult than of naturally mated queens for as yet unclear reasons. Other than that, artificially inseminated queens behave normally and are able to produce large colonies (Baer & Schmid-Hempel 2000). Note that *B. terrestris* queens need to hibernate before being able to found a colony, which in the vast majority of cases starts within the first four to eight weeks post-hibernation.

We inseminated a total of 297 queens. Queens had to be used as they became available, but inseminations were systematically alternated such that each patriline was equally represented within each matriline. The number of queens surviving hibernation for each of the matrilines was 6, 8, 14, 14, 15, 55, 75 and 97, respectively. Therefore, only 13 queens died during hibernation, but neither matriline nor patriline had an influence on hibernation success. The number of males per patriline (as represented by their sperm) was 53, 55, 57, 59 and 60.

After hibernation, queens were kept singly and their survival was monitored with regular censuses every 1–3 days up to 100 days post-hibernation. Those still alive after a previously chosen limit of 100 days were removed and entered as censored data into the survival analysis. Queens of *B. terrestris* typically initiate a colony within one to two months at the latest, whereas queens that fail to start a colony within this period can safely be considered as having failed (P. Korner and P. Schmid-Hempel, personal observation). We also recorded whether the queen produced offspring. Colonies still growing on day 100 were allowed to finish their colony cycle and their fitness was recorded as the number of sexuals (males and young queens) produced. A pragmatic fitness measure was used (the sum of males plus two times the young queens (see Baer & Schmid-Hempel 1999)) but the qualitative conclusions do not depend on the precise fitness measure used.

The length of the radial wing cell of males was taken as a measure of male size. The radial wing cell is known to correlate with wing size (Schmid-Hempel & Schmid-Hempel 1996), which correlates with wet weight (Müller *et al.* 1996). The males were immediately frozen after their sperm had been taken and their thoraxes stored at −20 °C. In January 2003, thoraxes were homogenized in 300 µl of cacodylic buffer to measure the antibacterial activity of the male's haemolymph as a measure of immune status. We took this measure as an indicator for the possible presence of a sexually transmitted infection, which may have been responsible for the observed patriline effect. For this purpose, 2 µl of supernatant (centrifugation at 6500 rpm for 10 min, 4 °C) were placed into holes (2 mm in diameter) punched into agar containing a test bacterium (*Arthrobacter globiformis* from Institut Pasteur Paris, no. 81.84 T; 10^5 bacteria per ml agar, 6 ml of bacteria–agar solution in Petri dishes with a diameter of 9 cm). After incubation for 24 h at 37 °C, a zone of inhibition can be observed around the supernatant, where no bacteria grow. The diameter of this clear zone was size-corrected (diameter/male size^3)

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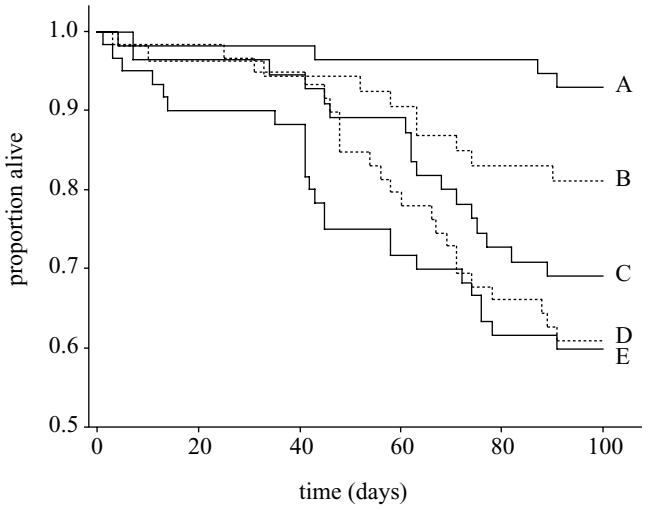

Figure 1. Observed survival of queens inseminated with five different patrilines (A–E; sample sizes are 57, 53, 55, 59 and 60, respectively). Patrilines B and D are dotted for better readability. Patriline had a significant effect on queen survival (see § 3 for statistics).

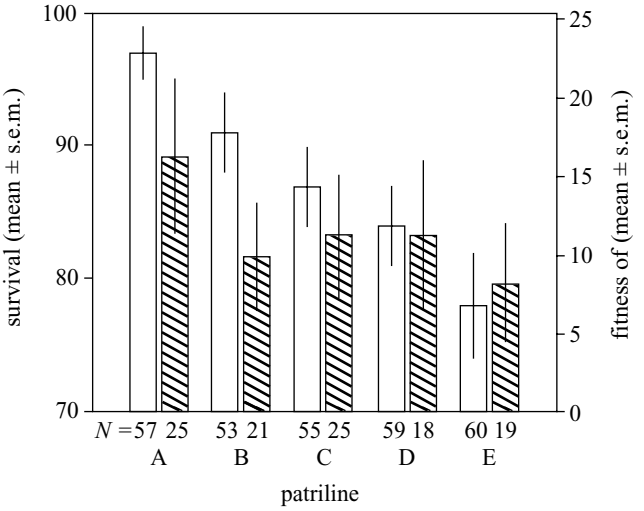

Figure 2. Queen survival (open bars, left axis; all queens included), defined as days after hibernation (Kaplan–Meier estimate of mean survival; censoring at day 100) and queen (her colony's) fitness (hatched bars, right axis; only queens included that had at least one offspring), defined as the number of male offspring plus two times the number of queen offspring. Queens were inseminated with male sperm from the five patrilines A–E. Sample sizes are given below the bars, and letters indicate the same patrilines as in figure 1.

and taken as the measure of antibacterial activity. For statistical analysis we used R1.6.1 (survival analysis) and SPSS11 for Macintosh (other analysis).

3. RESULTS

Post-hibernation lifespan was strongly influenced by patriline and also by matriline (figure 1; Cox regression model: $n = 284$, patriline $p = 0.001$, matriline $p = 0.02$). A possible interaction between patriline and matriline could not be tested with a Cox regression because the model coefficients did not converge. However, the interaction term did not significantly improve a binary logistic regression model predicting whether the queen survived to the end of the experiment (i.e. for at least 100 days) or not ($n = 284$, interaction term $p \approx 1$). Also, for the queens that died early (i.e. before day 100), the lifetime was not significantly predicted by the interaction term (ANOVA: $F_{6,47} = 0.07$; $p = 0.998$; only matrilines and patrilines with $n \geqslant 8$ included). The date of insemination ($p = 0.7$), sperm collection score ($p = 0.25$), size of the male ($p = 0.13$) and antibacterial activity of the male ($p = 0.25$) did not significantly improve the Cox regression model.

Only 15% of queens that did not survive to 100 days founded a colony before this time (defined as the production of at least one offspring). By contrast, 51% of queens surviving to day 100 founded a colony, thus reinforcing the importance of queen survival for eventual fitness. The likelihood of colony foundation was neither affected by patriline (logistic regression: $n = 284$, $p = 0.99$), nor by matriline ($p = 0.64$) or a patriline × matriline interaction ($p \approx 1$). Size and antibacterial activity of male, date of insemination and sperm collection score all had no significant effect on colony foundation probability (logistic regression: $n = 166$; antibacterial activity, insemination date and sperm collection score: $p > 0.8$, size $p = 0.18$).

The sperm collection score therefore did not explain the differences in female survival or, for that matter, colony success. It was, however, affected by patriline (multinomial logistic regression: $\chi^2_{28} = 70.5$, $p < 0.001$), but not by size of the male (same analysis: $\chi^2_7 = 6.6$, $p = 0.47$). This suggests that some physical properties of the sperm (including possible additional substances within the accessory testes) differed among patrilines.

We found that insemination with sperm of certain patrilines reduces the survival of queens. Overall, this negative effect might be compensated for if the surviving queens carrying sperm of such patrilines had a better colony performance. To check for this compensatory effect, we plotted the mean fitness of each patriline for queens that actually founded a colony (figure 2, open bars) next to mean survival of all queens (figure 2, hatched bars). Figure 2 does not support the idea of such a compensation, as the patriline associated with the highest mean queen survival (i.e. patriline A, figures 1 and 2) also produced the highest fitness in the surviving queens that had a colony. Vice versa, patriline E (associated with lowest mean survival) was also associated with the lowest performance of the surviving colony-founding queens (figure 2).

4. DISCUSSION

We found a very significant effect of patriline on female survival in a singly mated species after artificial insemination with sperm only (this may include hitherto unknown additional substances in minute quantities from the accessory testes but not from the accessory glands). The finding is remarkable, because, so far, an effect on female survival of the male ejaculate is primarily known for multiply mated species, and the effect has been attributed to accessory gland products rather than sperm. In the context of a habitually singly mated species it is more difficult to see how reduced longevity could be selected for as both male and female have a common fitness interest. However, decreased longevity could result as a correlated response to the male's ability to prevent the female from a second

mating (e.g. Sauter *et al.* 2001). This remains untested so far. We discuss our findings in the light of three hypotheses: genetic incompatibility, transmission of products influencing queen behaviour and transmission of disease.

(a) *Genetic incompatibility*

If genetic incompatibility were responsible for the differential survival of queens we would expect to find a patriline × matriline interaction effect. Our data give no indication of such an effect. Therefore, a patriline detrimental for one matriline was also detrimental for the others. At least as far as our experimental data goes, genetic incompatibility does not seem to be the cause of the observed pattern. However, three of 155 inspected spermatheca (the female organ where the sperm is stored) showed some evidence of melanization, suggesting that an immune reaction had occurred (Ashida & Brey 1998). This might indicate the presence of some kind of somatic–genetic incompatibility (Bishop *et al.* 1996), a hypothesis that deserves further investigation.

(b) *Transmission of products influencing queen behaviour*

It is interesting to see a strong patriline effect on the sperm collection score. This means that the sperm from some patrilines was easier to collect than from others, and it suggests that sperm consistency (which is important for the collection process itself) differed among patrilines. However, we found no statistically significant effect of this score on female longevity or reproductive output. Also, it seems unlikely that queens were sperm-limited, as only *ca*. 10% of the available sperm of a male is transferred to the female in natural matings (Baer & Schmid-Hempel 2000).

With the sperm we may perhaps have transferred substances that influence life-history parameters of the queen. For example, decisions on the timing of hibernation events (start, duration) may be affected by such substances and vary among patrilines. Our artificial hibernation may then have differentially influenced the queens according to patriline. Insect males also transfer substances to stimulate female reproductive behaviour (Chapman 2001; Wolfner 2002). In these cases, increased reproductive activity is likely to be associated with increased female mortality, a disadvantage that would then be compensated for by higher fecundity of the surviving queens. But as figure 2 shows, our current data do not support this stimulation hypothesis. Also, substances that influence female behaviour have so far mainly been found in the accessory glands (Baer *et al.* 2000, 2001) and not in the accessory testes, from where we collected the sperm.

(c) *Transmission of 'disease'*

The differential effect on queen survival could also occur if we transferred a cryptic disease, such as a bacterium or virus, together with the sperm of the males. For this purpose, we measured the antibacterial activity of the male thoraxes. Infections cause a strong induction of this activity in insects (Hetru *et al.* 1998), and, therefore, the observed activity could at least give an indication for a possible transfer of pre-existing infections. In our data, however, there was no correlation between male antibacterial activity and queen lifetime or reproductive parameters. This, of course, does not totally exclude the possibility that pathogens were transmitted with the sperm but at least narrows the range of possibilities.

5. CONCLUSION

The observed influence of patriline on queen longevity is an observed fact but its causes remain elusive. However, these results suggest that the influence of males on female longevity is neither limited to multiply mating animals, nor to accessory gland products.

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