

## Research article

# Colony success of the bumble bee, *Bombus terrestris*, in relation to infections by two protozoan parasites, *Crithidia bombi* and *Nosema bombi*

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**Summary.** *Crithidia bombi* is a prevalent endoparasite of bumblebees that is transmitted both horizontally between and vertically within colonies of its host, the bumble bee *Bombus terrestris*, and to the next generation. By experimentally infecting or not infecting laboratory-raised colonies with a standard inoculum before their transfer to the field, this study was aimed at evaluating the level of virulence of *C. bombi* under natural conditions. However, an unexpected finding was a substantial and seasonal increase in infections of natural populations, such that all colonies quickly became infected once exposed to the field. On average, experimentally infected colonies showed positive signs of infection 9.5 days after being exposed, not different from the 11.7 days in untreated colonies. Not surprisingly therefore, no significant differences between the two experimental groups in measures of colony success, such as male and young queen production or time of emergence of sexuals, were found. Overall though, *C. bombi* showed low levels of virulence which fits recent models for parasites with correlated horizontal and vertical transmission rates. On the other hand, the number of sexuals produced depended on the length of time over which reproduction could be sustained. Thus, early colonies and those with large first brood and large maximum size were at an advantage. Also, strong effects of site occurred. In addition, many colonies became naturally infected with the microsporidian *Nosema bombi*. Such infections were not associated with experimental treatment or colony size, but correlated with an increased production of sexuals, particularly males.

**Key words:** Parasite, host reproductive success, virulence, colony size, *Bombus terrestris*, *Crithidia bombi*, *Nosema bombi*.

## Introduction

Theory predicts that parasites should evolve to a level of pathogenicity that maximises their fitness. In particular, it is expected that parasites with high rates of horizontal transmission should evolve towards higher levels of pathogenicity (e.g. Bull, 1994; Ebert and Herre, 1996). However, when pathogenicity is maintained by a correlation with horizontal transmission rates, increased levels of vertical transmission lead to lower expected levels of virulence (Lipsitch et al., 1996). In this study we investigated whether the pathogenic effects of the intestinal parasite *Crithidia bombi* (Trypanosomatidae, Zoomastigophorea, Lipa and Triggiani, 1980) on its host, the bumble bee *Bombus terrestris* L., match this prediction by comparing the reproductive success of experimentally infected and untreated colonies in the field. *C. bombi* is a common parasite with observed prevalences of around 10–20% of workers or more (Shykoff and Schmid-Hempel, 1991a). It is transmitted horizontally between colonies when workers of uninfected colonies ingest parasite cells from flowers previously visited by infected workers (Durrer and Schmid-Hempel, 1994). Cells are passed on to other colony members (within-colony transmission) and eventually to gynes (young queens), which ensures vertical transmission of the parasite into the next generation. In addition, Wu, (1994) found multiple infections inside spring-queens. Both, the occurrence of multiple infections and high horizontal transmission rates, would predict *C. bombi* to be a parasite with strong pathogenic effects (Bull, 1994).

According to existing knowledge, the effects of *C. bombi* are varied and subtle, a pattern that is characteristic for insect-pathogenic trypanosomes in general (Schaub, 1994). For example, when infected and uninfected bumble bee workers are sampled in the field, infected workers carry pollen less often than uninfected ones (Shykoff and Schmid-Hempel, 1991a). Furthermore, laboratory-kept colonies infected with *C. bombi* have lower growth rates early in their cycle (Shykoff and Schmid-Hempel, 1991b), a phase that is

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crucial for eventual colony success in the field (Müller and Schmid-Hempel, 1993). Infected bees have smaller ovaries (Shykoﬀ and Schmid-Hempel, 1991b), smaller fat bodies and slightly higher mortality rates (Schmid-Hempel and Huck, in prep.). In this study, we tested the effect of *C. bombi* with experimentally infected colonies under field conditions. However, the results also yielded insight into the force of infection and determinants of reproductive success.

## Materials and methods

We reared a set of 32 colonies in the laboratory from hibernated queens of *Bombus terrestris* collected in spring 1995 around Zürich, Switzerland. At any one time, the so raised colonies could be ranked in size, i.e. number of workers, on a given day. As soon as both of the two largest colonies in the set had reached a size of at least seven workers, we arbitrarily selected the larger colony of this pair and infected half of its workers with a standard inoculum of *C. bombi*. Workers were chosen at random by flipping a coin. In the next round of picking – from the remaining set of colonies – the pair of largest colonies meeting the minimum-size requirement, the smaller of the two largest colonies would be inoculated, and so forth, to balance colony size between treatments. The mean size of all colonies at this point was  $12.9 \pm 0.84$  (S.E.) workers. The inoculum was prepared from a mixture of two strains of *C. bombi* from our lab-cultures, initially isolated from host populations in the Jura mountains (Wu, 1994). The infected colony was then assigned to group “Inoculated”. The other colony of the pair was not infected and assigned to group “Untreated”. To expose these colonies to the field situation, we chose one of three field sites around Zürich (“Wynegg”, “Wesendonck”, “Fluntern”) and transferred the pair there (“Date of transfer”). Choice of field site was systematically varied, such that the first pair was transferred to Wynegg, the second to Wesendonck, and so forth, until all were placed in the field. The first colonies were placed in the field on May 15, the last ones on July 7, 1995. The mean Julian day of field transfer was day 163.6 (3.01, S.E.) (equivalent to 13 June). Colony size on the day of field transfer was noted (“Initial size”) and used as a covariate in the analyses.

In the field, we checked the colonies every fourth day. On these occasions, we sampled faeces from four randomly chosen workers out of each colony to check for cells of *Crithidia bombi* and spores of *Nosema bombi*. In addition, after the start of the reproductive phase, the produced males and young queens (gynes) were collected and fresh body mass (mg) measured. From these data, we estimated the following measures for each colony: (1) Colony fitness,  $W$ , calculated with the Shaw-Mohler-formula (e.g. Charnov, 1982) as  $W = m/M + f/F$ , where  $m$ ,  $f$  = number of males and females (gynes) produced in the colony, and  $M$ ,  $F$  = total number of males, gynes produced in the entire experimental population. Fitness values were arcsin-transformed to normalise variances for analysis. (2) Delay to reproduction for males and gynes, as days post field-transfer until the first males or gynes eclosed inside the colony. (3) The length of the reproductive period for males and gynes, as days from eclosion of the first to eclosion of the last male or gyne.

Gynes collected from colonies were transferred to the laboratory, where they were kept under standard conditions with food ad libitum in separate groups per colony and date of collection. All gynes of a colony reaching the age of ten days were brought together with twice as many males in flight cages. For this, males from five additional laboratory colonies were randomly chosen by coin flipping. These colonies were started in the same way from queens from the same area as the queens of the experimental colonies. The flight cages were then observed and mating success, i.e. whether a mating occurred or not, recorded per individual queen. Mating success could only be observed for six colonies. Data analyses were done with SPSS 6.1. The five gynes that were infected with *N. bombi* were excluded from the analysis of mating success. Values are means and S.E. if not indicated otherwise.

## Results

A striking finding of our experiment was that both groups of colonies, i.e. those from Inoculated and Untreated, quickly showed signs of infection with *C. bombi* after they were exposed to the field. In fact, workers of all colonies, except one (from the untreated group), shed cells of *C. bombi* in their faeces on average  $10.55 \pm 1.15$  days ( $n = 31$ ) post transfer (Table 1). An ANCOVA for this delay to infection (in days post field transfer) with the two covariates Transfer date and Initial colony size yielded no significant effects for factor treatment, site or interaction, but a significant negative relationship ( $\beta = -0.487 \pm 0.06$ ) with the covariate Transfer date (Table 1). In fact, the later in the season a colony was transferred to the field, the shorter was the delay to the first infected workers inside (Fig. 1).

Altogether, ten inoculated (62.5%,  $n = 16$ ) and seven untreated (43.8%,  $n = 16$ ) colonies reproduced ( $\chi^2 = 1.129$ ,  $P = 0.29$ ). Calculated fitness per colony was on average  $W = 0.06 \pm 0.02$  (units,  $n = 32$ ). An ANCOVA for fitness (Table 1) with the two covariates Transfer date and Initial colony size yielded no significant effects for factor treatment or interaction, but a highly significant effect of site and a positive relationship with the covariate Initial colony size ( $\beta = 0.381 \pm 0.001$ ). Transfer date had no relationship to fitness. Fitness was higher at field-site Fluntern than at the other two field-sites (Table 1; Fig. 2). In addition, colony fitness was positively correlated with maximum colony size achieved (Fig. 2;  $F_{1,30} = 18.53$ ,  $P < 0.001$ ) and with the delay to this maximum, i.e. the number of days post field-placement when maximum colony size was reached ( $F_{1,30} = 5.597$ ,  $P = 0.025$ ).

Nine of the inoculated and six untreated colonies produced males. On average, males were produced  $39.93 \pm 3.42$  days ( $n = 15$  colonies) post field-transfer (Table 1). This delay was related to the date when the colony was transferred to the field, such that earlier colonies produced males over a longer period of time (Table 1; pairwise correlation:  $r = -0.765$ ,  $P = 0.001$ ;  $n = 15$  colonies). As expected from the randomised assignment of colonies, neither the duration of the reproductive period for males, i.e. from eclosion of first to last male, nor the number of males produced per colony differed among treatments or among sites (Table 1). However, the number of produced males was positively correlated with the length of the reproductive period for males (Fig. 3a).

Queens were only produced in four inoculated and three untreated colonies (i.e. in 21.9% of the  $n = 32$  colonies), and they eclosed on average  $38.29 \pm 5.53$  days post field-transfer ( $n = 7$  colonies). Again, early colonies produced gynes somewhat earlier (Table 1; pairwise correlation:  $r = -0.697$ ,  $P = 0.082$ ,  $n = 7$  colonies) and the length of the reproductive period was positively correlated with the number of gynes produced (Fig. 3b). Unfortunately, too few queens emerged to fully analyse the effects of treatment or site (Table 1).

Because exposed colonies quickly became infected in the field, we could not find a lasting difference in the prevalence of infection between colonies from the Inoculated (average prevalence =  $69.1 \pm 3.6\%$ , S.E.) and Untreated