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Abstract In social insects, the reproductive strategies adopted by colonies emerge as a complex property of individual behaviours, but as yet we are often unable to fully explain them in evolutionary terms. In bumblebees, colonies adopt either a short-lived strategy specializing in male production, or a longer-lived strategy in which mainly new queens are produced, but this results in males emerging long before mates are available; this strategy can only easily be explained if older males are at a significant reproductive advantage. Here we examine how age and morphological characters influence mating success of male bumblebees. In two separate experiments in which single virgin males and females were confined together, we found that young males and heavy males mated more swiftly and copulated for less time compared to old males or lighter males, respectively. However, in competitive situations age proved to be unimportant and the only factors to influence mating success were the lengths of the fore and hind tibiae, with strong directional selection for long leg length. Fore and hind legs are both used in courtship, so both traits are associated with plausible mechanisms under selection. It has previously been argued that, in times of food stress, bumblebee colonies should produce males as male size is less likely to be strongly correlated with fitness than female size. Our results suggest that this may not be so, since aspects of male size directly impact on their mating success. Our results leave unexplained the emergence of males many days before new queens.

Keywords Sexual selection · aging · leg length · mate choice · colony-level strategies · *Bombus* · apoidea

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Introduction

How fitness changes with age is an intriguing question for a number of reasons. Much of this research is focussed on actuarial and functional senescence and the evolutionary processes that may drive these phenomena, such as mutation accumulation or antagonistic pleiotropy (Medawar 1952; Williams 1957). Studies of changes in reproductive performance with age have attracted special attention because of additional evolutionary explanations that relate to sexual selection (Bonduriansky et al. 2008). Age-based indicator mechanisms are postulated to explain female preferences for older males (who have by virtue of their old age proven their ability to survive in the current environment, and therefore represent males of high genetic quality; Brooks and Kemp 2001). Although there are several theoretical and empirical studies supporting these mechanisms (e.g. Kokko 1998; Pike et al. 2007; Zuk 1987), the relative importance of age-based mate choice for indirect benefits remains uncertain, in part because it is often difficult to rule out confounding factors associated with age (such as paternal ability) that might drive female preferences. There are also empirical and theoretical examples of situations in which females prefer younger or intermediately aged males (Ritchie et al. 1995; Jones et al. 2000; Beck and Powell 2000). Moreover, system specific complexities may cause age-related mate choice for reasons that are rather unrelated to good-genes sexual selection in the strict sense, such as inbreeding avoidance (De Luca and Cocroft 2008; Keller et al. 2008).

In addition to the above-mentioned complexities, evaluating how one particular aspect of a male's phenotype, such as age, influences his mating success is difficult because phenotypic characters are usually intercorrelated, and sexual selection rarely occurs in a single episode. There are many studies of how male characters covary with mating success, but relatively few of these focus on how male traits affect events occurring before courtship (e.g., long-distance attraction; Brooks et al. 2005) and after copulation (e.g., post-copulatory sexual selection; Bussière et al. 2006). Selection in these episodes need not concur in sign or magnitude with selection on courtship for mating success (Danielsson 2001), and consequently it is often hard to evaluate whether selection for pair formation is representative of sexual selection as whole Hunt et al. 2009.

Bumblebee males have features that make them less vulnerable to many of these problems. First, most morphological characters involve sclerotized structures which do not change with bumblebee age, and can therefore be assessed relatively independently of it. In most species, female bumblebees are not known to remate in the field (Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000; Paxton et al. 2001; Payne et al. 2003) and so post-copulatory sexual selection and any potential interaction between sperm performance and ageing (Radwan 2003; Reinhardt 2007) are unlikely to play a substantial role in selecting on male traits. Furthermore, since male bumblebees acquire most of their developmental resources thanks to the efforts of their nursing sisters, their early developmental progress probably depends less on their own morphology than is the case for many other species. Notwithstanding some understudied aspects of male patrolling and pheromone communication (Baer

2003; Goulson 2010), these facts together suggest that measures of selection based on mating success are much more likely to represent the total intensity of sexual selection in male bumblebees than for most other insect species in which male morphology is more constrained by selection for resource acquisition and success in sperm competition.

At a colony level, there are a number of aspects of the reproductive strategies adopted by bumblebees that are not yet fully understood. Firstly, they generally seem to have a highly male-biased sex-ratio which cannot readily be explained, particularly since kin-selection theory predicts that, in monogamous species such as most bumblebees, workers should favour a 3:1 investment in females relative to males (while the queen should favour equal investment in each) (reviewed in Bourke 1997; Goulson 2010). Secondly, observations of the timing of production of males versus new queens in bumblebee nests suggest that males are produced considerably earlier than one might expect, unless females show a strong preference for old males. Protandry appears to be the norm in bumblebees (reviewed by Bourke 1997) and many other insects, and is thought to optimise the number of virgin females that a male will encounter during his life. However, based on studies of *B. terrestris*, it seems that bumblebee colonies vary greatly in the timing of their reproductive phase (Duchateau and Velthuis 1988): some colonies switch from rearing workers to rearing reproductives while the colony is still small, approximately 10 days after emergence of the first workers. In contrast other colonies switch later, an average of 24 days after emergence of the first workers. Early-switching colonies produce mainly males, and late-switching colonies produce mainly new queens. Because males take ~26 days to develop from egg to adult, while queens take ~30 days (Duchateau and Velthuis 1988), on average, the first males produced by early-switching colonies become adult 18 days before the first new queens emerge. Males are fully fertile and reproductively active after just 5 days (Greeff and Schmid-Hempel 2008), and leave the nest. There is, therefore, no clear explanation as to why males are produced so early, since they then have to survive for many days before they are likely to get a chance to mate, a period during which they could succumb to predation or disease.

One possibility is that older males have a mating advantage that selects for early male development. Alternatively, it has been suggested that colonies switch early and specialize in male production in response to stress or low resource availability. Beekman and Van Stratum (1998) argue that this strategy is adaptive if male size is less closely linked to reproductive success than is female size. This hypothesis seems plausible since small queens suffer high mortality in hibernation (Beekman et al. 1998). However, the relationship between male size and reproductive success has not been investigated.

Here we study the relationships between male morphology, male age and mating success under a range of experimental conditions. We test how age and mass affect the speed with which a male bumblebee mates, the duration of mating and his propensity to mate a second time. We also perform experiments to determine the influence on mating success of the phenotype in a multivariate context. This experiment allowed randomly selected males to compete for single females, and assessed the relative importance of several different aspects of the phenotype once correlated characters were controlled for statistically.

Methods

Study Insects and Rearing Conditions

A total of 32 *B. terrestris* colonies were imported from Koppert UK limited, and the colonies were allowed to produce sexuals (new queens and males) in a 24-hour dark climate room at 28±1°C, 50±5% RH. We used red light for observations and animal husbandry. The bee diet consisted of *ad libitum* pollen and sugar solution. The newly emerged sexuals were removed daily and kept in nestmate groups in mesh screen cages (30×30×30 cm) with a typical number of 50 males or 20 queens in each cage. We stored male and queen cages in separate rooms at 25±1°C and 50±5% RH with *ad libitum* pollen and sugar solutions until individuals were used in experiments. Thirty-one colonies produced males and 29 produced queens, and a total of 2,164 queens and 3,571 males were harvested.

Mating Conditions

Mating experiments were carried out in a climate room at 25±1°C and 50±5% RH. All matings were staged in transparent plastic boxes (10×8×8 cm) which had mesh screens for ventilation. Observations were made under white light (Philips MCFE65-80 W/29, Poland) positioned 0.5 m above the mating cages. Queens and males present in a mating cage were from different colonies.

Effect of Male age on Mating Behaviour

This experiment was done with four different age groups of males viz. 6, 9, 12, and 15 days old post-eclosion, with 7 day old queens. In each mating cage a virgin queen and a virgin male were allowed to interact freely. If the couple had not started mating within 1 h, the trial was aborted and the pair was discarded. For trials in which mating occurred, we recorded the mating latency (time elapsed between the introduction of male and female into the mating cage until initiation of copulation) and the duration of mating (time elapsed between copulation initiation to termination by each pair). As soon as they completed mating, the mated queen was taken out and replaced with a virgin queen, and the new pair were observed for the next hour. The remating latency (time elapsed between the introduction of the second queen into the mating cage until initiation of copulation with her), and the duration of the second copulation were also recorded. If any male did not remate within 1 h then the pair was excluded from analyses of remating behaviour. For each age group, we conducted trials until 20 males had mated once. All of the mated males were used for remating. The number of males which mated a second time within 1 h were $n=17$, 17, 15 and 15, for the age groups 6, 9, 12 and 15 days-old, respectively.

Effect of Male Mass on Mating Behaviour

To observe the effect of male body mass on mating behaviour we conducted a separate experiment using 7 day-old queens and 7 day-old males, which are at the peak of receptivity for mating (Duchateau and Marien 1995). We measured male body mass using a digital top loader balance (Fisher Scientific SG-202, LA NORME NMB003, DU Canada) to an accuracy of 0.01 g. Mass was measured immediately before mating trials took place. We placed a virgin female together with a virgin male and recorded mating latency, mating duration, remating latency and remating duration as described above. Data were collected from a total of 105 mated males of which 84 mated twice.

Effect of Sex Ratio on Mating Behaviour

To test how the number of competing males for each queen in the mating cage affects mating behaviour, we observed mating in female: male sex ratios of 1:5, 1:10, 1:15 and 1:20. All males and queens were 7 day old virgins, and 16–17 replicates were carried out for each sex ratio. For each queen to male ratio, the animals were allowed to interact in the mating cage freely for 1 h, and the above methods were employed for estimating mating latency and duration.

Male Phenotype and Mating Success Under Competition

Because our previous experiments revealed intriguing effects of age and mass on mating behaviour, we sought to determine the relative impact of phenotypic traits (including age) on male mating success in a competitive context. We conducted a series of mate choice trials using virgin males of varying age and morphology. Males were between 7 and 24 days old and the queens were all 7 days old. Each male was used only once, and was randomly paired with a rival (except that no siblings were used in the same trial). A total of 406 males and 203 queens were used. We observed mating by placing one virgin queen and two randomly selected males that were weighed immediately prior to mating trials in a mating cage (10 × 8 × 8 cm); one male was given a spot (Nice day, Euro Office Ltd. London) on the thorax so that they could be distinguished. Males and queens were allowed 1 h to mate; if no mating occurred within 1 h they were discarded. After the completion of mating, the bees were killed by freezing, and several morphological characters were subsequently measured. We weighed all bees on an analytical precision balance (Denver Pinnacle Series 0.04 Digital, BCBP 100, Germany). We removed wings and all legs from the bees, and photographed them using a binocular microscope connected to a digital camera (Moticam 2000, 2.0 M Pixel USB2.0, China). We measured the length of wings and all tibiae (see Supplementary Figs. 1a and b) using Motic Images Plus software (Version 2.0, Novex, Holland).

Statistical Analysis

An analysis of variance (one way ANOVA) with post hoc Duncan's Multiple Range Test (DMRT) was applied to compare the mating behaviour of different age groups of males. Comparisons were made between mating and remating latency, and duration between first and second mating for each age group using Student's t-tests (independent samples). The effect of queen to male ratio on mating latency and duration was analysed by one way ANOVA with post hoc Duncan's Multiple Range Test. We used a Pearson's correlation to examine the relationship between body mass and mating latency and duration. All the above analyses were performed using SPSS (PASW Statistics 17).

For the analysis of sexual selection on male phenotypes, we used standard linear and nonlinear approaches for estimating selection gradients (Arnold and Wade 1984a, 1984b). The parameter estimates we report are derived from standard regressions, but the p-values associated with these parameters are derived from logistic regressions because the response variable is binomial. We doubled quadratic coefficients when reporting quadratic selection gradients as recommended by Stinchcombe et al. (2008). Because our design featured two males in every trial, the outcome for each male was not independent of the outcome for his partner. We therefore used bootstrap resampling methods as suggested by Mitchell-Olds and Shaw (1987) to test for bias in our estimated gradients based on the full sample of males. In each iteration of this procedure, we sampled one male from each trial, selected at random, and executed the regression analysis to generate an unbiased estimate of the true parameters. We then repeated the resampling procedure 10,000 times and observed the distribution of parameter estimates to generate 95% confidence intervals for the true parameters. These analyses were all performed using R version 2.10.1 (R Development Core Team 2009). We visualized selection on individual phenotypic traits using nonparametric cubic splines created in the vegan package (Oksanen et al. 2009) of R software.

Because the standard multiple regression approach can systematically underestimate the strength of nonlinear selection on phenotypes, we also used a canonical rotation of the γ matrix of nonlinear selection gradients (Phillips and Arnold 1989; Blows and Brooks 2003) to isolate the axes of multivariate phenotypic space along which nonlinear selection was strongest. This allowed us to assess with more confidence whether age was important in a multivariate nonlinear context. We visualized the two statistically significant axes of this nonlinear selection (which were also the axes along which nonlinear selection was strongest) using a nonparametric thin-plate spline. We modelled the spline using the fields package (Fields Development Team 2006) of R software, adopting the smoothing parameter (lambda) that minimized the generalised cross-validation (GCV) score.

Results

Effect of Male Age on Mating Behaviour

In all the age groups of males, some started mating within 1 min of their introduction into the mating cage, and the number of matings increased with time (Fig. 1). The mean mating latency varied from 11.1±12.1 min for 6 day old males to 22.9±17.1 min for 15 day old males, with age having a significant positive effect on mating latency (mean±SD, ANOVA: $F_{3,\ 76}=2.73$, $p<0.05$). The mean remating latency also varied significantly with age, with older males remating after a longer interval (Fig. 1, $F_{3,\ 60}=2.77$, $p<0.05$). There was no significant difference in mating latency for first and second matings ($t_{35}=0.27$, $p=0.90$; $t_{35}=0.29$, $p=0.31$; $t_{33}=0.33$, $p=0.28$; $t_{33}=0.25$, $p=0.56$ for age group 6, 9, 12 and 15 days, respectively). Mating duration also differed between age groups, being longer in older males in both their

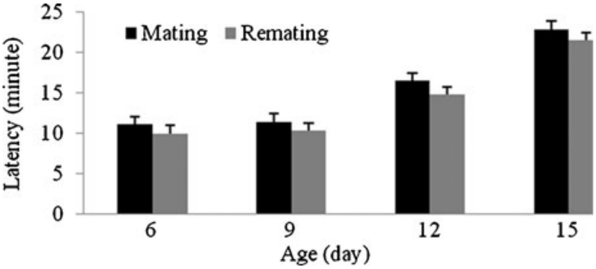

Fig. 1 Effect of male age on the mating and remating latency (mean±se) of *B. terrestris*. Mating and remating latency both increased significantly with age ($F_{3,\ 76}=2.73$, $p<0.05$ and $F_{3,\ 60}=2.77$, $p<0.05$, respectively)

first mating ($F_{3,\ 76}=2.68$, $p<0.05$) and in their second (ANOVA: $F_{3,\ 60}=2.75$, $p<0.05$) (Fig. 2), and there was no significant differences between first and second mating durations ($t_{35}=0.89$, $p=0.13$; $t_{35}=0.67$, $p=0.53$; $t_{33}=1.42$, $p=0.66$; $t_{33}=0.67$, $p=0.72$ for age group 6, 9, 12 and 15 days, respectively).

Effect of Male Mass on Mating Behaviour

Heavier males tended to have lower mating latencies ($r=-0.231$, $p<0.05$, Fig. 3a). We found significant negative correlations between male body mass and remating latency ($r=-0.251$, $p<0.05$, Fig. 3b), mating duration ($r=-0.402$, $p<0.01$, Fig. 3c) and remating duration ($r=-0.346$, $p<0.01$, Fig. 3d).

Effect of Sex Ratio on Mating Behaviour

The sex ratio in the mating cages significantly affected mating latency; it was significantly longer at high male:queen ratios compared to low ratios (ANOVA: $F_{3,\ 62}=3.20$, $p<0.05$) but mating duration was not significantly different (ANOVA: $F_{3,\ 62}=1.52$, $p=0.21$) (Fig. 4).

Sexual Selection on Age and Morphology

Table 1 lists the linear and nonlinear selection gradients estimated via these mating trials. The bootstrapped 95% confidence regions around these gradients (unbiased by the nonindependence of males in the same mating trial, see Methods) are indicated below the selection gradients in Table 1 of the online supplementary material.

Our multivariate analysis revealed significant linear selection for larger fore- and hind-tibiae, but no significant selection on mass or age once other aspects of the phenotype were accounted for (Table 1). There was little evidence of quadratic or

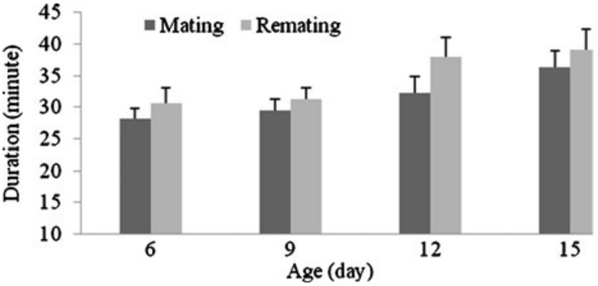

Fig. 2 Effect of male age on the mating and remating duration (mean±se) of *B. terrestris*. Mating and remating duration both increased significantly with age ($F_{3,\ 76}=2.68$, $p<0.05$ and$F_{3,\ 60}=2.75$, $p<0.05$, respectively)

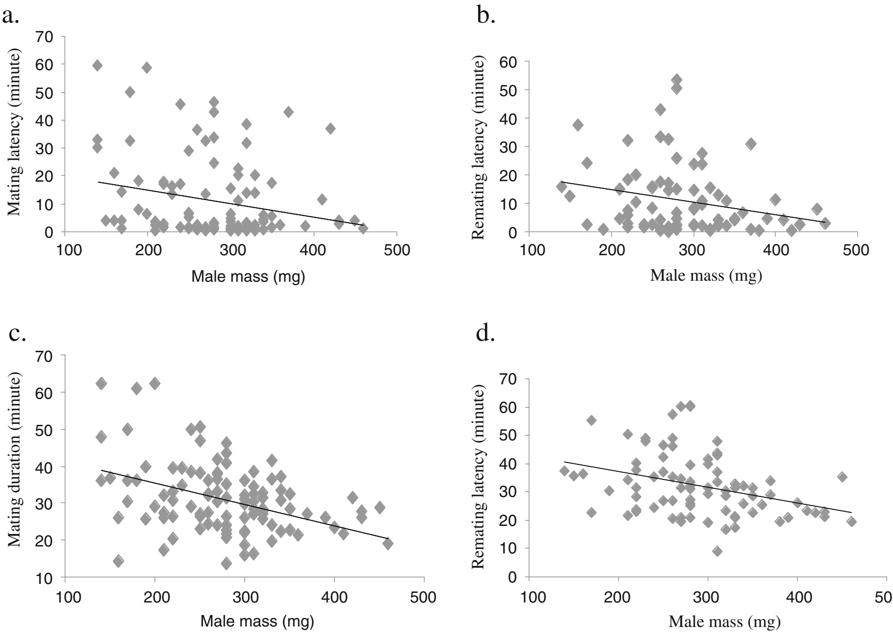

Fig. 3 Relationship between male body mass and **a** mating latency, **b** remating latency, **c** Mating duration and **d** Remating duration of *B. terrestris*. All four relationships are significant (r=−0.231, $p<0.05$; r=−0.251, $p<0.05$; r=−0.402, $p<0.01$; r=−0.346, $p<0.01$)

correlational selection on morphology, with only two of the 21 nonlinear terms in the regression approaching significance (both $P<0.1$; Table 1). Neither of these terms featured age; instead they represented the cross products of mass and hind tibia length, and wing length and mid tibia length.

To further explore nonlinear selection in our data set, we conducted a canonical rotation of the γ matrix of nonlinear selection gradients to isolate the main multivariate axes of nonlinear selection (Phillips and Arnold 1989). This procedure revealed two axes of multivariate phenotypic space that were significantly related to fitness, one of which was primarily disruptive, and the other primarily stabilizing (the details of this canoncial analysis are provided in Table 2 of the online supplementary material). Age did not load strongly on either of the two axes representing most of the nonlinear selection.

As a final confirmatory step, we visualized selection on leg lengths and male age using nonparametric splines that were not constrained to fit straight lines. The linear selection on male leg lengths was clearly evident in these plots, and present both in

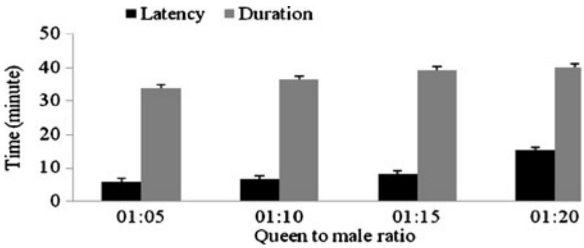

Fig. 4 Effect of queen to male ratio on mating latency and duration of *B. terrestris* (mean±se). Latency increased when more males were present ($F_{3,62}=3.20$, $p<0.05$) but mating duration did not change significantly ($F_{3,62}=1.52$, $p=0.21$)

Table 1 The vector of standardized linear selection gradients (β) plus the bootstrapped confidence intervals for them, and the matrix of quadratic and correlational selection gradients (γ) on male bumblebee age and morphology. The confidence intervals around the quadratic and correlational selection gradients are provided in Table 1 of the online supplementary material. Randomization probabilities: ° $P<0.1$; * $P<0.05$; ** $P<0.01$

Trait	β	γ					
		Age	Mass	Wing length	Fore tibia length	Mid tibia length	Hind-tibia length
Age	−0.017	0.017					
Mass	−0.040	−0.060	−0.072				
Wing length	−0.002	0.012	−0.070	0.082			
Fore tibia length	0.124**	0.041	−0.011	−0.057	0.067		
Mid tibia length	0.047	0.011	−0.003	0.125°	−0.055	−0.036	
Hind tibia length	0.096*	0.004	0.126°	−0.078	0.112°	−0.040	−0.063

the raw data (morphological traits plotted against mating success, Fig. 5) and in partial plots illustrating selection once other traits were taken into account (not shown). By contrast, there was no hint of any directional or nonlinear selection on age in spite of the findings described above related to mating latency (Fig. 5). A thin-plate spline visualization of the nonlinear selection suggested by our canonical analysis revealed that this nonlinear selection was mainly due to minor curvature in the fitness surface, rather than classically stabilizing or disruptive selection (see Fig. 2 of the online supplementary material).

Discussion

In early-switching nests (which produce most male bumblebees) males emerge ~18 days before queens emerge from late-switching nests, and one might therefore predict that older males have a mating advantage. However we found that when a single male and queen were enclosed together, older bumblebee males were slower to initiate mating and

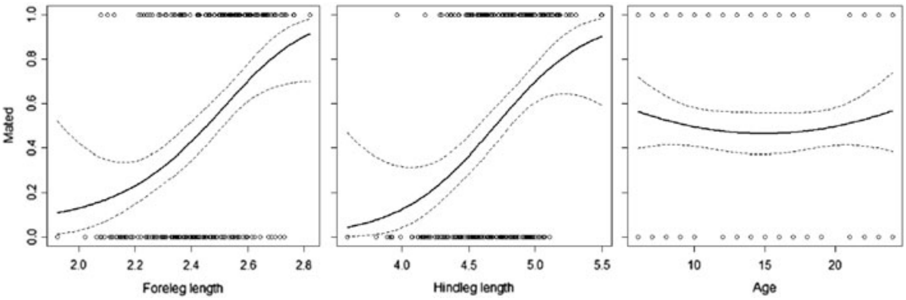

Fig. 5 Cubic splines illustrating positive linear sexual selection on fore tibia and hind tibia lengths in male *B. terrestris*, but no apparent sexual selection on age. These splines are on raw data rather than residuals, but the patterns for residuals are the same (data not shown)

copulated for longer, and that this pattern was repeated if males were given a chance to mate with a second queen. In separate experiments, male body mass was a significant predictor of the speed of initiation of mating and of copulation duration, with large males performing more quickly. Taken in isolation, these results would suggest that bumblebee nests should be under selective pressure to produce large males, and to produce them only a short time interval before the first virgin queens emerge (optimally about 5 days, the time it takes for males to mature and leave the nest, Greeff and Schmid-Hempel 2008). Our results therefore suggest that males from early-switching nests may be at a significant disadvantage in competition for mates with the smaller number of younger males produced from late-switching colonies.

Based on these data on mating latency, when two males were enclosed with a single female in a competitive situation, we predicted that younger, larger males would be most successful in obtaining mates. However, this was not the case; age did not predict mating success. The only factors which did influence mating success were the fore and hind tibia lengths (each contributing significant explanatory power to the model), with males having longer legs being more likely to mate. When two males were confined with a single virgin female, antagonistic interactions were frequent with the males often wrestling for position on top of the female and occassionally displacing each other. During courtship, male bumblebees climb on to the back of the female and grasp her thorax with their forelegs while stimulating her abdomen with their hind legs (MRA, pers. obs.). Males are considerably smaller than females, and relatively long legs may facilitate both gripping the female and stimulating her simultaneously. Of course it is also possible that long fore and hind legs are not themselves mechanistically responsible for the mating advantage we observed, and that instead some unmeasured trait or traits that covary strongly with the lengths of these legs mediates the outcome of sexual competition in bumblebees. It has been previously suggested that larger males may have larger genitalia that act as stronger forceps for attaching to the queen's sting apparatus (Williams 1985; Duvoisin et al. 1999). In the dung fly *Scatophaga stercoraria*, Simmons and Parker (1992) reported that larger males are able to pull the aculeus of the queen more forcefully and hence mated more quickly. However, we did not investigate the dimensions of the male genitalia, and how they covary with leg lengths is unknown.

The magnitude of directional selection we report is slightly less than the mean of values commonly reported for selection in nature (Kingsolver et al. 2001), and the fact that selection is rather weak may explain the persistence of variation in male performance traits even if these traits feature substantial genetic variation. Alternatively, it is plausible that balancing selection within or across the sexes acts to maintain variation in leg lengths among male bees. For example, leg length may negatively influence aerodynamics, which may be important in male patrolling behaviour (reviewed in Goulson 2010), or it may be that longer-legged males may be more susceptible to becoming caught in spider's webs. Given that they may have to survive for several weeks in the field before they encounter a mate, any factors increasing mortality could readily offset a mating advantage. At present we have no information on the heritability of leg length in bumblebees, so there is a clear need for further research in this area.

With regard to the reproductive strategies adopted by bumblebee nests, our results do not explain why early-switching colonies produce males long before queens are likely to be available to be mated, but they do illustrate that older males (up to 24 days) may suffer no disadvantage when competing with young males. They also somewhat call into question the argument that male size is less closely linked to reproductive success than is female size (Beekman and Van Stratum 1998). The evidence to date suggests that size is likely to be positively correlated with reproductive success in both sexes, but in which it has the strongest relationship remains unknown. In the context of the more general question concerning evolutionary explanations for reproductive ageing, our data suggest caution in interpreting associations between male age and reproductive success based on no choice trials or without appropriate consideration of possibly confounding correlated phenotypic traits. More studies adopting a multivariate perspective are needed, ideally featuring realistic conditions that simulate natural levels of mate competition.

The copulation durations observed in our study are similar to those reported in previous studies (e.g. Roseler 1973; Amin et al. 2009), with a mean duration of copulation varying from ~28 to 36 min for the youngest and oldest bees, respectively. Duvoisin et al. (1999) observed the copulation behaviour of *B. terrestris* using 10 day old queens and 15 day old males and reported that copulations lasted on average 37 min but that most sperm were transferred into the queen genital tract within the first 2 min. The remaining time in copulation may be required for transfer of the 'mating plug', a sticky substance that completely fills the queen bursa copulatrix after mating and prevents remating (Baer et al. 2000, 2001). Males transfer the mating plug to the female within 10–30 min of the onset of copulation (Duvoisin et al. 1999). The differences of mating duration among the age groups in our study may be due to differences in the speed of sperm and mating plug migration, or to differences in the amount of material transferred. It seems intuitive that older males who take longer to initiate mating may transfer sperm more slowly, but in the beetle *Propylea dissecta* older males mate for longer but transfer more sperm than younger males (Pervez et al. 2004). In agreement with our finding of a significantly longer copulation duration when mating for a second time, Roseler (1973) reported that *B. terrestris* males may mate up to eight times, and show an increase in copulation duration across subsequent matings. Similar patterns have been observed in other insects (e.g. Hughes and Chang 2000).

Our studies of mating latency in relation to sex ratio are of some practical relevance to those wishing to induce mating in bumblebees, for they suggest that intermediate ratios of 1:5–1:10 females to males produce the most rapid onset of copulation. The results are also interesting from a behavioural viewpoint; when only a single male is present, mating latency can be prolonged often because the male is inactive. The presence of other males seems to stimulate activity (MRA, pers. obs.). However, when large numbers of males are present, latency increases. In this situation several males are usually attempting to mate with the female simultaneously, and it seems likely that the increase in mating latency is due to interference between males.

There have now been many studies of the mating behaviour of *B. terrestris*, and of colony-level reproductive strategies. Some aspects remain poorly understood, notably the male-biased sex ratio, the factors which determine whether nests adopt an early or late-switching strategy, and why early-switching nests produce males long before there ought to be mates for them to copulate with. However, our understanding may be hampered by the heavy bias towards lab studies which may not accurately reflect the situation in the field; the reproductive strategies adopted by natural, wild nests have rarely been studied, and mating behaviour is exceedingly difficult to study under natural conditions, providing substantial challenges for future researchers.

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References

Amin MR, Than KK, Kwon YJ (2009) Copulation duration of bumblebee *Bombus terrestris* (hymenoptera: apidae): impacts on polyandry and colony parameters. J Asia-Pacific Entomol 12:141–144

Arnold SJ, Wade MJ (1984a) On the measurement of natural and sexual selection: theory. Evolution 38:709–719

Arnold SJ, Wade MJ (1984b) On the measurement of natural and sexual selection: theory. Evolution 38:720–734

Baer B (2003) Bumblebees as model organisms to study male sexual selection in social insects. Behav Ecol Sociobiol 54:521–533

Baer B, Maile R, Schmid-Hempel P, Morgan ED, Jones GR (2000) Chemistry of a mating plug in bumblebees. J Chem Ecol 26:1869–1875

Baer B, Morgan ED, Schmid-Hempel P (2001) A non-specific fatty acid within the bumblebee mating plug prevents females from remating. Proc Natl Acad Sci 98:3926–3928

Beck CW, Powell LA (2000) Evolution of female mate choice based on male age: are older males better mates? Evol Ecol Res 2:107–118

Beekman M, Van Stratum P (1998) Bumblebee sex ratios: why do bumblebees produce so many males? Proc R Soc Lond B 265:1535–1543

Beekman M, Van Stratum P, Lingeman R (1998) Diapause survival and post-diapause performance in bumblebee queens (*Bombus terrestris*). Ent Exp Appl 89:207–214

Blows MW, Brooks R (2003) Measuring non-linear selection. Am Nat 162:815–820

Bonduriansky R, Maklakov A, Zajitschek F, Brooks R (2008) Sexual selection, sexual conflict and the evolution of ageing and life span. Funct Ecol 22:443–453

Bourke AFG (1997) Sex ratios in bumblebees. Phil Trans R Soc Lond B 352:1921–1933

Brooks R, Kemp DJ (2001) Can older males deliver the good genes? Trends Ecol Evol 16:308–313

Brooks R, Hunt J, Blows MW, Smith MJ, Bussière LF, Jennions MD (2005) Experimental evidence for multivariate stabilizing sexual selection. Evolution 59:871–880

Bussière LF, Hunt J, Jennions MD, Brooks R (2006) Sexual conflict and cryptic female choice in the black field cricket, *Teleogryllus commodus*. Evolution 60:792–800

Danielsson I (2001) Antagonistic pre- and post-copulatory sexual selection on male body size in a water strider (*Gerris lacustris*). Proc Roy Soc Lond B 268:77–81

De Luca PA, Cocroft RB (2008) The effects of age and relatedness on mating patterns in thornbug treehoppers: inbreeding avoidance or inbreeding tolerance? Behav Ecol Sociobiol 62:1869–1875

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL http://www.R-project.org

Duchateau MJ, Marien J (1995) Sexual biology of haploid and diploid males in the bumble bee, *Bombus terrestris*. Insect Soc 42:255–266

Duchateau MJ, Velthuis HHW (1988) Development and reproductive strategies in *Bombus terrestris* colonies. Behaviour 107:186–207

Duvoisin N, Baer B, Schmid-Hempel P (1999) Sperm transfer and male competition in bumblebees. Anim Behav 58:743–749

Estoup A, Scholl A, Pouvreau A, Solignac M (1995) Monandry and polyandry in bumble bees (Hymenoptera, Bombinae) as evidenced by highly variable microsatellites. Mol Ecol 4:89–93

Fields Development Team (2006) Fields: tools for spatial data. National Center for At-mospheric Research, Boulder, CO. URL http://www.cgd.ucar.edu/Software/Fields

Goulson D (2010) Bumblebees; behaviour, ecology and conservation. Oxford University Press, Oxford

Greeff M, Schmid-Hempel P (2008) Sperm viability in the male accessory testes and female spermathecae of the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). Eur J Entomol 105:849–854

Hughes L, Chang BSW (2000) Effects of mating history on ejaculate size, fecundity, longevity, and copulation duration in the ant-tended lycaenid butterfly, *Jalmenus evagoras*. Behav Ecol Sociobiol 47:119–128

Hunt J, Breuker CJ, Sadowski JA, Moore AJ (2009) Male-male competition, female mate choice and their interaction: determining total sexual selection. J Evol Biol 22:13–26

Jones TM, Balmford A, Quinnell RJ (2000) Adaptive female choice for middle-aged mates in a lekking sandfly. Proc Roy Soc Lond B 267:681–686

Keller LF, Reid JM, Arcese P (2008) Testing evolutionary models of senescence in a natural population: age and inbreeding effects on fitness components in song sparrows. Proc Roy Soc Lond B 275:597–604

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P (2001) The strength of phenotypic selection in natural populations. Am Nat 157:245–261

Kokko H (1998) Good genes, old age and life-history trade-offs. Evol Ecol 12:739–750

Medawar PB (1952) An unsolved problem of biology. H.K. Lewis, London

Mitchell-Olds T, Shaw RG (1987) Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149–1161

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Stevens MHH, Wagner H (2009) Community ecology package. R package version 1.16-22/r912. http://R-Forge.R-project.org/projects/vegan/

Paxton RJ, Thoren PA, Estoup A, Tengo J (2001) Queen-worker conflict over male production and the sex ratio in a facultatively polyandrous bumblebee, *Bombus hypnorum*: the consequences of nest usurpation. Mol Ecol 10:2489–2498

Payne CM, Laverty TM, Lachance MA (2003) The frequency of multiple paternity in bumble bee (*Bombus*) colonies based on microsatellite DNA at the B10 locus. Insect Soc 50:375–378

Pervez A, Omkar, Richmond AS (2004) The influence of age on reproductive performance of the predatory ladybird beetle, *Propylea dissecta*. J Insect Sci 4:22

Phillips PC, Arnold SJ (1989) Visualizing multivariate selection. Evolution 43:1209–1222

Pike TW, Blount JD, Bjerkeng B, Lindstrom J, Metcalfe NB (2007) Carotenoids, oxidative stress and female mating preference for longer lived males. Proc Roy Soc Lond B 274:1591–1596

Radwan J (2003) Male age, germline mutations and the benefits of polyandry. Ecol Lett 6:581–586

Reinhardt K (2007) Evolutionary consequences of sperm cell aging. Q Rev Biol 82:375–393

Ritchie MG, Couzin ID, Snedden WA (1995) What's in a song—female bushcrickets discriminate against the song of older males. Proc Roy Soc Lond B 262:21–27

Roseler PF (1973) Die Anzahl der Spermien in Receptaculum seminis von Hummel Koniginnen (Hym. Apoidea, Bombinae). Apidologie 4:267–274

Schmid-Hempel R, Schmid-Hempel P (2000) Female mating frequencies in *Bombus* spp. from Central Europe. Insect Soc 47:36–41

Simmons LW, Parker GA (1992) Individual variation in sperm competition success of yellow dung flies, *Scatophaga stercoraria*. Evolution 46:366–375

Stinchcombe JR, Agrawal AF, Hohenlohe PA, Arnold SJ, Blows MW (2008) Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution 62:2435–2440

Williams GC (1957) Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411

Williams PH (1985) A preliminary cladistic investigation of relationships among bumblebees (Hymenoptera, Apidae). Syst Entomol 10:239–255

Zuk M (1987) Variability in attractiveness of male field crickets (Orthoptera: Gryllidae) to females. Anim Behav 35:1240–1248