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To cite this version:

Mark J.F. Brown, Boris Baer. The evolutionary significance of long copulation duration in bumble bees. Apidologie, 2005, 36 (2), pp.157-167. hal-00892132

HAL Id: hal-00892132

https://hal.science/hal-00892132v1

Submitted on 11 May 2020

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Apidologie 36 (2005) 157–167
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DOI: 10.1051/apido:2005008

Review article

The evolutionary significance of long copulation duration in bumble bees[1]

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Received 10 October 2004 – Revised 22 November 2004 – Accepted 24 November 2004

Published online 1 June 2005

Abstract – Copulation duration – the time spent in copula – is of particular interest in social insects. Female social insects (or queens) generally only mate during a short, initial post-emergence period, and in the absence of extensive pre- or post-copulatory mate guarding, copulation is the only point in time at which the sexes directly interact with each other. Although copulation duration is likely to be under natural selection, because queens depend upon successfully transferred sperm, longer copulation durations offer males the possibility to manipulate paternity in their own interest. Consequently, copulation duration might be one of the few traits in social insects where sexual selection has resulted in evolutionary conflict between the sexes. Here we review the available data on copulation duration in bumble bees and, by relating it to other aspects of mating in *Bombus*, develop a framework within which we may understand the selective forces that have shaped this enigmatic behaviour.

***Bombus* / mating / sperm transfer / mating plug / polyandry / sexual selection**

1. INTRODUCTION

Mating in animals is a complex process, involving numerous pre- and post-copulatory behaviours (Eberhard, 1985; Eberhard and Cordero, 1995; Birkhead and Moller, 1998; Simmons, 2001; Baer, 2003; Boomsma et al., 2005). A key feature of mating is the actual amount of time animals spend in copula. While copulation initially evolved to facilitate sperm transfer, it also provides an arena for sexual conflict, and the duration of copulation will reflect these two forces (Simmons, 2001). Copulation entails costs, both physiological, e.g., the amount of energy put into copulation itself, and ecological, e.g., an enhanced exposure to parasitism or predation. This latter cost may be especially important in social insects, where the main defensive weapon, the sting, is immobilised during copulation, and where copulating pairs have greatly reduced mobility. Consequently, one might expect copulation to take up only that amount of time required for the successful transfer of sperm. This seems to be the case in honey bees, where copulation is over in a matter of seconds (Koeniger and Koeniger, 1991; Winston, 1991), and stingless bees, where matings take less than a minute (Engels and Engels, 1988). Nevertheless, although copulation duration is therefore expected to be relatively short, males may be selected to prolong the period in copula to enable them to guard females in response to the elevated risk of sperm competition due to queen remating (Simmons, 2001). Furthermore, prolonged copulation behaviour allows males to manipulate females, either mechanically with their sexual organs (Eberhard, 1985) or chemically with gland compounds transferred during or shortly after sperm transfer (Simmons, 2001).

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[1] Manuscript editor: Gudrun Koeniger

Table I. Copulation duration in bumble bees. Data shown are the sample size, mean copulation duration and range of copulation durations (where available). Data for *Bombus ardens* are observations which stopped prior to the end of copulations, and for *B. lapidarius* are of unknown provenance (the author states that in normal cases the pair remain in copula for three hours) but are included for completeness.

Subgenus	Species	N	Mean Duration (min)	Range (min-max) or SD (±)	Source
Bombus ss	*lucorum*	15	29	16–40	Rutrecht and Brown, unpublished data
Bombus ss	*terrestris*	7	36.2	± 12.1	Röseler, 1973
		93	36.9	15–75	Duvoisin et al., 1999
Cullumanobombus	*rufocinctus*	19	9	?	Foster, 1992
Fervidobombus	*atratus*	95	25.3	5–60	Garofalo et al., 1986
Fervidobombus	*californicus*	51	44.66	?	Foster, 1992
Melanobombus	*lapidarius*	?	180	?	Postner, 1953
Pyrobombus	*ardens*	3	>36	3 records, all incomplete (i) >32, (ii) >28, (iii) >48	Katayama, 1964
Pyrobombus	*bifarius*	16	42.19	?	Foster, 1992
Pyrobombus	*frigidus*	16	10.19	?	Foster, 1992
Pyrobombus	*hypnorum*	-	-	25–55	Pouvreau, 1963
		12	24.7	±6.5	Röseler, 1973
		64	26.3	6–75	Brown et al., 2002 (Swiss)
		82	31.6	10–86	Brown et al., 2002 (Swedish)
Pyrobombus	*pratorum*	1	~90	-	Cumber, 1953
		6	?	?–60	van Honk et al., 1978
		2	37.5	30–45	Rutrecht and Brown, unpublished data

In contrast to honey bees and stingless bees, bumble bees have been shown to have much longer copulation durations (Foster, 1992; Duvoisin et al., 1999; Brown et al., 2002). While mate guarding has been suggested as a potential reason for extended copulation (Foster, 1992; Duvoisin et al., 1999), no comprehensive attempt has been made to analyse variation among and within species in copulation duration and its relation to other features of mating in bumble bees. Given that copulation duration is a feature of mating that appears to be under solely male control (Baer, 2003), such an analysis may provide important insights into the role of males in the evolution of mating strategies in bees. In this paper, we conduct the first examination of inter- and intra-specific variation in copulation duration in bumble bees, and suggest useful directions for future research.

2. SHOULD I STAY OR SHOULD I GO?

A summary of our current knowledge of copulation duration in bumble bees is presented in Table I. All of these data are based on matings in the laboratory, or in semi-natural flight cages. However, given the readiness with which male bumble bees attempt to copulate under laboratory conditions, it seems unlikely that natural copulations would differ in terms of copulation duration.

Generally, the mean copulation duration for bumble bee species is substantially longer than

for honey bees and stingless bees, and appears to be much longer than would be expected if there were no advantages to staying in copula. Out of the nine species for which we have estimates for mean copulation duration (values for *B. ardens* and *lapidarius* are not means), only two have a mean copulation duration of less than 25 minutes. Copulation durations reported for the two remaining species in the table were both longer than 45 minutes. Across all species, mean copulation duration varies almost 5-fold, and excluding the two outlier species (*frigidus* and *rufocinctus*) with unusually short mean copulation durations, more than 1.5-fold. Thus, at the interspecific level we are faced with two questions: (1) why is copulation duration so long in bumble bees, and (2) what explains the variation among species in copulation duration? We address these questions in the first section below.

In addition to interspecific variation in copulation duration, there is a large amount of variation across males within a species as to how long they remain in copula. For example, in *B. hypnorum* copulations range from six to 86 minutes in length, a 14-fold (or, within a single population, 12.5-fold) difference (Tab. I). While intra- and interspecific variation are likely to be related, we deal with the problem of intraspecific variation in a separate second section.

3. WHY IS COPULATION DURATION SO LONG IN BUMBLE BEES?

3.1. Sperm transfer

A simple explanation for long copulation durations in bumble bees might be that sperm transfer from the male to the female takes an equally long time. This is a functional explanation, implying that copulation has evolved solely under natural selection for efficient sperm transfer. However, the available data strongly suggest that this is not the case. Duvoisin et al. (1999) found that sperm transfer took between 30 and 120 s in *B. terrestris*, in contrast to a mean copulation duration of 36.9 minutes. Given the anatomical similarity of *Bombus* species, there is no good *a priori* reason to suggest any great difference in sperm transfer rates across species. Thus sperm transfer seems unlikely to explain copulation duration data in bumble bees.

3.2. Sperm migration

After deposition in the *bursa copulatrix* of a female, sperm has to migrate to the spermatheca (Duvoisin et al., 1999). Again, the only data we have are from *B. terrestris* where Duvoisin et al. (1999) showed that, on average, 50% of the sperm have migrated into the spermatheca by about 43 minutes after the start of copulation. This is 6–7 minutes after the end of the average copulation (see Tab. I). There are at least two potential reasons that copulation duration and sperm migration might be linked. Firstly, the initiation of migration may require the continued presence of the male. A feature of bumble bee copulation is the continuous abdominal contractions of the attached male, long after transfer of the sperm and mating plug (see below) has been accomplished. Such contractions may produce a physical stimulus for sperm migration from the *bursa copulatrix* to the spermatheca. Secondly, if a subsequent second mating could interfere with sperm migration (Sauter et al., 2001), the extended copulation might be explained as mate guarding to facilitate successful filling of the spermatheca. However, the mean copulation duration in *B. terrestris* is shorter than the time required for complete sperm migration (see above). Thus, sperm migration is unlikely to completely explain long copulation durations across *Bombus*.

3.3. Mating plug dynamics

One of the most exciting recent discoveries in bumble bee mating biology was the existence of mating plugs that chemically inhibit queens of *B. terrestris* from remating (Duvoisin et al., 1999; Baer et al., 2000, 2001; Sauter and Brown, 2001; Sauter et al., 2001; Baer, 2003). Mating plugs are produced in the accessory glands and have also been found in *B. hypnorum* (Brown et al., 2002) and *B. lucorum* (Baer, unpublished data). Despite the fact that males in the polyandrous species *B. hypnorum* would be expected to invest less in producing a mating plug (which is no longer efficacious in controlling female mating

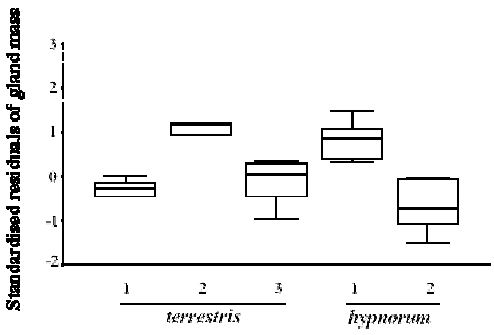

Figure 1. Colony-level variation in male investment into accessory glands. The data shown are 3 colonies of *B. terrestris* and 2 of *hypnorum*. There was no effect of species on residual gland mass (nested ANOVA: $F_{1,23} = 0.02$, $P > 0.05$), but there was a significant effect of colony (nested ANOVA: $F_{1,23} = 4.16$, $P < 0.025$).

behaviour) and more into sperm production (see Baer and Boomsma, 2004), males of *hypnorum* and *terrestris* appear to have similar investment into the plug-producing accessory glands (Fig. 1). However, it should be noted that mating plugs deteriorate more rapidly in polyandrous *B. hypnorum* queens than in *B. terrestris* queens (Brown et al., 2002), which might imply that the chemical composition of the mating plug differs between these species (but see Mikheyev, 2003). The presence of plugs in two widely separated subgenera suggests that they are likely to occur throughout the genus and mating plugs have even been hypothesized to be widespread in social insects in general (Boomsma et al., 2005). In *B. terrestris* the mating plug is completely transferred within about 10 minutes (Duvoisin et al., 1999) and thus transfer alone cannot be an explanation for prolonged copulation duration. Because males are capable of moving previously-placed sperm and mating plugs when copulating with a non-virgin queen (Sauter et al., 2001), it also seems unlikely that prolonged copulations are designed to enhance the functionality of plugs, despite the fact that males clearly face the risk of getting their sperm displaced by competitors. However, if plug chemicals require some time in which to take physiological effect, long copulation durations may still play a role in facilitating mating plug function.

3.4. Tentative conclusions

From this survey, it appears that the facilitation of sperm migration into the spermatheca, whether physically, chemically or by mate guarding, is the most likely reason for long copulations across bumble bee species. However, this is not a complete explanation. Firstly, as noted above, copulation duration does not match the amount of time required to completely fill the spermatheca. It is possible that the initial movement of sperm into the spermatheca is the most important step to safeguard male reproductive success, but we can think of no a priori reason as to why this should be true. Secondly, at least two species of bumble bee have dramatically shorter copulation durations (Tab. I). Investigation of the rates of sperm migration in these two species could be a crucial test of the idea that copulation duration is related to the successful migration of sperm into the spermatheca.

4. DIFFERENT STROKES: WHAT EXPLAINS THE VARIATION AMONG SPECIES IN COPULATION DURATION?

4.1. Sperm transfer, sperm migration and mating plug transfer

The discussion above suggests that differences in sperm migration rates among species might explain variation in the mean copulation duration of different species (Tab. I). Similarly, the possible absence of a mating plug in *B. frigidus* and *rufocinctus* might explain their anomalously short copulation durations. However, species-level differences in mating behaviour may also play an important role.

4.2. Pre-mating behaviour

Bumble bee species have different male pre-mating strategies (Alford, 1975; Baer, 2003). These include perching (where males rest on a perch and then fly out and engage prospective mating partners), nest-guarding (where males search for and stake-out conspecific nests and attempt to mate with virgin queens when they leave the nest), territoriality, and flight-paths (where males scent mark routes and patrol

Table II. Relation between body size and copulation duration across species. L = body length, W = wingspan, r = rank (large, 1, to small, 4, across the four species), R = mean rank across all body size measurements, CD = species ranked by copulation duration (long to short; see Tab. I). All L and W measurements are in mm.

	Male				Queen				Ratio (Q:M)					
Bombus	L	r	W	r	L	r	W	r	L	r	W	r	R	CD
terrestris	15	1	31.5	1	21.5	1	40.5	1	1.43	1	1.29	1	1	2
lucorum	15	1	31	2	19.5	2	37.5	2	1.3	3	1.21	2	2	3/4
hypnorum	15	1	30	3	18.5	3	36.5	3	1.23	4	1.22	3	2.83	3/4
pratorum	12	4	24.5	4	16	4	30	4	1.33	2	1.22	3	3.5	1

them). These strategies clearly vary in their physiological cost, with the flight-path strategy probably being the most energetically expensive, and thus might be expected to trade-off with other costly mating activities such as copulation duration. However, of the eleven species in Table I, only *B. rufocinctus* and *B. californicus* do not follow the flight path strategy, and these two species cover the range of mean copulation durations. Consequently, from the present data it seems unlikely that pre-mating behaviour influences copulation duration, but further studies are clearly needed.

4.3. Monandry vs. polyandry

Both Foster (1992) and Brown et al. (2002) suggested that copulation duration might be related to the occurrence of multiple mating. Based on observational data of mating rates, Foster (1992) showed that for the North American species *rufocinctus*, *californicus*, *bifarius* and *frigidus* there was a strong positive correlation between copulation duration and frequency of multiple mating. Foster argued that this might indicate mate-guarding by males to prevent queens from remating (Simmons, 2001). In contrast, the polyandrous European *hypnorum* has the shortest copulation duration of the four European species, and Brown et al. (2002) argued that the reduced cost of shorter copulations might explain the existence of polyandry in this species. This explanation was supported by data showing that the shorter a queen's first copulation, the more likely she was to mate for a second time. In fact, it seems logical that shorter copulation durations are more likely to be found in polyandrous species. Since mate-guarding is obviously ineffective in polyandrous species, males are expected to reduce reproductive investment in copulation duration and instead attempt to gain additional copulations. In contrast, if mate-guarding is effective, then species with long copulation durations should be associated with monandry (as the genetic data for the European species shows (Schmid-Hempel and Schmid-Hempel, 2000)). As acknowledged by Foster (1992), observational data on copulation frequencies are an inaccurate measure of actual mating frequencies in natural populations (Boomsma and Ratnieks, 1996), and because even normally monandrous species will remate under artificial conditions (Sauter et al., 2001; personal observations), it is unclear whether the positive correlation between copulation duration and mating rate in North American species is spurious.

4.4. Body size

Despite their morphological similarity, bumble bee species do vary strongly in size. If the energy costs of copulation, or the physical effects of abdominal contractions (assuming that these play a role in effective copulation – see above) scale non-linearly, then they might explain cross-species variation in copulation duration. Size can be measured in a number of ways, either as male size, queen size, or the ratio of the two. In Table II we present data calculated from von Hagen (2003) for the four European bumble bee species for which we know mean copulation duration. While this is a small sample size, the data give no support to the idea that there is a correlation between any measure of body size and the length of time that species spend in copulation.

4.5. Tentative conclusions

Given our current state of (or lack of!) knowledge, the two most likely explanations for species-level variation in copulation duration seem to be the mate-guarding hypothesis and its relation to multiple mating, and the sperm migration hypothesis. These are not mutually exclusive explanations, and they could in fact be working together or even against each other in the evolution of copulation duration. For example, if males are selected to reduce time spent in copula but sperm migration requires extended copulation, the actual copulation duration will be some trade-off between the two.

5. NATURAL VIAGRA: WHAT CAUSES INTRASPECIFIC VARIATION IN COPULATION DURATION?

The huge amount of variation across males within a species in how long they spend in copula demands an explanation. Simple explanations might be that this variation reflects differences among males in age, male quality, or previous mating experience (see below). However, in the experiments of Duvoisin et al. (1999) and Brown et al. (2002) males were age-controlled, fed ad libitum from larvae to sexual maturity, and virgin. So, what other factors might determine the duration of an individual copulation?

5.1. Sperm migration

Sperm migration has already been implicated as a possible cause for the classically long bumble bee copulation. In addition, data on sperm migration also showed significant variation among copulations (Duvoisin et al., 1999). For example, of the 24 queens who had their copulation terminated between 20 and 60 min after the start of copulation, 33% showed no evidence of sperm migration, whilst 17% already had a filled spermatheca. Furthermore, the first full spermatheca was seen at 30 min after the start of copulation, whilst unfilled spermathecae could still be found after 70 min. In the absence of correlational data between copulation duration and sperm migration rate, it is impossible to say whether males time their copulation duration to enable complete sperm migration. However, it is certainly possible that copulation duration may be related to sperm migration. Males may remain in copula in order to prevent matings with a second male from disrupting sperm migration (Sauter et al., 2001), and differences among males in sperm migration rate might result in differences among males in copulation duration.

5.2. Male quality

While male quality – as measured by age, size, immunocompetence or colony-of-origin – is unlikely to explain differences among species in copulation duration, it might easily play an important role in determining variation among males within a species. As has already been mentioned, males vary among colonies in their investment into the glands responsible for mating plug production (Fig. 1), Baer (2003) found significant variation across males within and among colonies in sperm length in three *Bombus* species, and Tasei et al. (1998) found substantial variation in the number of sperm virgin males possess in their accessory testes, ranging from 4 000 to 230 000. Males also vary greatly in size (personal observation). In *B. atratus* larger males, unsurprisingly, have more sperm (Garofalo et al., 1986). Unfortunately, no study has quantified the relationship between any of these factors and copulation duration, although there is good reason to hypothesise that these factors might have a substantial influence. For example, males possessing few sperm might invest more into mate-guarding or gland compounds, to maximise their chances of securing sole paternity. Similarly, males who transfer the chemicals needed to prevent queens from remating at a low rate may need to remain in copula longer to inhibit remating to the same degree as a male who can rapidly transfer large amounts of the inhibitory chemicals.

Male quality may also vary with experience. In both *B. hypnorum* and *B. atratus*, the number of sperm transferred on average by a male decreases through his 1st to 3rd copulation (Tab. III; Röseler, 1973; Garofalo et al., 1986). This is inversely related to copulation duration, which increases with the number of matings

Table III. The relation between number of copulations, sperm transfer and copulation duration. Data shown are mean ± SD (sample size) for time in minutes and number of sperm, and for *B. terrestris* and *hypnorum* are from Röseler (1973) and for *B. atratus* are from Garofalo et al. (1986).

	1st mating	2nd mating	3rd mating	4th mating
Bombus	copulation duration			
atratus	19.17 ± 12.42 (6)	31.83 ± 19.6 (6)	40 ± 43.59 (3)	30 (1)
hypnorum	24.7 ± 6.5 (12)	29.6 ± 12.3 (11)	41.1 ± 14.6 (8)	-
terrestris	36.2 ± 21.1 (7)	45.7 ± 16.6 (7)	78 (1)	-
	sperm transferred			
atratus	49 000 ± 15 130 (6)	30 500 ± 10 930 (6)	22 666 ± 6 027 (3)	24 000 (1)
hypnorum	13 500 ± 9 500 (12)	12 300 ± 5 600 (11)	7 200 ± 4 400 (8)	-
terrestris	43 300 ± 30 000 (7)	40 600 ± 32 100 (7)	53 100 (1)	-

(Tab. III). While *B. terrestris* exhibits no change in sperm transfer from one copulation to the next, males show a similar increase in copulation duration across subsequent matings (Tab. III; Röseler, 1973). Thus, it seems that males invest more into mate-guarding-type behaviour with each copulation. This makes sense if males value copulations with respect to their relative investment. Given that bumble bee males cannot make new sperm after maturation, each time they ejaculate their remaining sperm become relatively more valuable. Consequently, to maximise their reproductive fitness we would expect males to invest more into protecting later copulations, which is what we see in all three species described above (Tab. III). An alternative explanation might be that it takes longer to transfer sperm and mating plug material with each copulation, perhaps due to a decline in available supplies. However, there are as yet no data to support either contention.

5.3. An honest signal?

If copulation duration reflects male quality, as measured by fitness or in terms of the amount of sperm transferred, and as it is energetically costly to the male, it may act as an honest signal of male quality. Data from the polyandrous *B. hypnorum* (Brown et al., 2002) initially supported this idea. In this species, queens are more likely to accept a second mate the shorter their first copulation was. The authors interpreted this from the perspective of a queen minimizing her own energetic or time cost of mating. However, it could also be interpreted as queens using copulation duration as a signal of male quality, and re-mating on the basis of this signal. But how reliable is copulation duration as an honest signal? Given that copulation duration increases with an apparent decrease in male quality (see above, Tab. III), it would appear that if *B. hypnorum* queens are using copulation duration as a signal, then they are making non-adaptive decisions as re-mating is least likely when the 1st copulation took place with a low quality, non-virgin male.

5.4. Reaction to female status?

So far, we have mostly discussed copulation duration as if it were a fixed trait of the male. However, data from *B. hypnorum* (Brown et al., 2002) suggest that males can actually make decisions about how long to remain in copula based on the intrinsic value of the copulation event. In their experiments, Brown et al. found that when a queen mated for a second time, the duration of copulation was significantly shorter than that for first matings. As copulation duration is controlled by males, and as males available for second matings were virgin and of the same age as males used for first matings, these data suggest that males were able to assess the already mated status of the females and reduce their energetic investment into copulation. Given that males have a lower fitness return from a doubly-mated queen (Baer and Schmid-Hempel, 2001), such a strategy makes evolutionary sense. How males recognise female status remains unknown, although it may depend upon chemoreceptors in the male genitalia.

5.5. Tentative conclusions

While factors such as size, age and experience may all play a role in generating variation among males in copulation duration, current data indicate that other factors must be at least as important. Again, sperm migration may explain some of this variation, but the fact that males can control the duration of copulation suggests that this trait should be viewed from a context-dependent perspective.

6. CONCLUSIONS AND SUGGESTIONS FOR A RESEARCH PROGRAM

We suggest that the problem of explaining copulation duration in bumble bees needs to be approached at three different levels. While explanations for generally long copulations in *Bombus*, species-level variation in this trait, and intraspecific variability may be related, there are also likely to be factors, e.g., male size, which have explanatory power at only one of these levels. Given that our current state of knowledge is preliminary at best, and given that this behaviour deserves explanation, what might be the best routes down which to direct future work?

As we have shown, sperm migration rates and mate-guarding seem the most likely explanation for long copulations at the genus level. The role of sperm migration rate could be tested quite simply. By conducting a series of laboratory matings and splitting queens into two groups, one where males are removed after sperm transfer and the other where they are left undisturbed, and flash-freezing queens at regular time-points after the onset of copulation, it should be possible to determine whether male presence is necessary for, or enhances sperm migration. Further experiments to test the mate-guarding hypothesis could involve artificial insemination techniques (Baer and Schmid-Hempel, 2000), for example, by placing sperm in the *bursa copulatrix* of females and then testing for effects on sperm migration in the absence and presence of a subsequent copulation. The sperm present in the spermatheca could then be genotyped using quantitative PCR to determine how each set of sperm contributed to successful insemination of the female. If mate-guarding is important we would expect to see less of the artificially inseminated sperm in the spermatheca of subsequently mated females.

At the species level, sperm migration rates, the presence or absence of a sperm plug, and the mating system (monandry vs. polyandry) may all have an influence on variation in copulation duration. While sperm migration rate and the existence of sperm plugs should be examined in those species where we already know copulation duration, our greatest need here is for new comparative studies. Given the ease with which bumble bees can be reared and mated in laboratory settings, we would encourage researchers to examine copulation duration, sperm migration rates and sperm plug dynamics in the 19 species for which we already know the mating system (Estoup et al., 1995; Schmid-Hempel and Schmid-Hempel, 2000; Payne et al., 2003).

Interestingly, for one species, *B. hypnorum*, we have data on copulation duration for several geographically disjunct population (Tab. I). It appears that Swedish populations may have longer copulation durations than populations from further south in Germany and Switzerland. We speculate that it would be worthwhile looking for correlates of this variation, for example, differences in predation pressure (the ecological cost of mating) or in levels of multiple mating (the evolutionary benefit of mate-guarding). While current data are sparse, there is at least the suggestion of a similar cline in mating frequency, with northern populations having higher levels of multiple mating (Schmid-Hempel and Schmid-Hempel, 2000; Paxton et al., 2001; but see Brown et al., 2003).

The individual level of variation is perhaps the easiest one to approach experimentally. At present, nothing is known about the role of male size, nutritional status or age in determining copulation duration, and all of these features could easily be measured and manipulated in a laboratory setting. Correlations between sperm migration rates and individual copulation duration could again be tested using a separate-and-freeze protocol (Duvoisin et al., 1999, see above). Finally, the ability of males to change copulation duration based on queen status and the potential for copulation duration to serve as a signal should be ascertained in other polyandrous and monandrous species. Last male

precedence might explain shorter copulation duration times for second males (as seen in *B. hypnorum*), and so it would be interesting to study the relative contribution of different males to offspring of multiply-mated queens based on the order of mating.

The role of males in the mating biology of social insects has been neglected for too long (Baer, 2003). Copulation duration in bumble bees represents an easily defined and potentially costly and important trait that is amenable to study and may shed great insight into the factors that have determined male mating behaviour in this genus. We believe that future studies of this trait and how it relates to other aspects of mating biology would be highly productive.

ACKNOWLEDGEMENTS

We would like to thank Gudrun Koeniger for inviting us to write this manuscript, and giving us the chance to develop our views on the role of copulation duration in the mating biology of bumble bees. The ideas within have been enhanced by critical discussion in the symposium "The neglected gender: biology of the male social bee" at the 1st European Conference of Apidology 2004, and by comments from two reviewers. Many thanks to Kazuki Tsuji and Johan Billen for accessing and translating references for us. MJFB was supported by grants EI-SC/2002/209 from the Enterprise Ireland Basic Research Grants Scheme and HEA/NSP Strand 1 from the Higher Education Authority North-South Programme for Collaborative Research. BB was supported by a SNF grant for advanced researchers PA00A-104991.

Résumé – La longue durée de copulation chez les bourdons : quelle signification du point de vue de l'évolution ? La durée de copulation – période durant laquelle un couple reste in copula – est un aspect central de l'accouplement chez les animaux. La longueur de la durée de copulation devrait être déterminée par une série de pressions de sélection, par exemple la sélection naturelle sur l'efficacité du transfert de sperme et la minimisation du temps et des coûts énergétiques de l'accouplement et par la sélection sexuelle des mâles qui maximalisent la paternité. Les abeilles mellifères (*Apis* spp) et les abeilles sans aiguillon (Meliponinae) ont des durées de copulation courtes, indiquant que la sélection naturelle prédomine chez ces groupes. Par contre, les bourdons (*Bombus* spp) ont des durées de copulation à la fois longues et très variables, ce qui suggère que la durée de copulation peut représenter un exemple de conflit évolutif entre les deux sexes. Nous avons analysé les données disponibles pour les bourdons à trois niveaux – interspécifique, intraspécifique et intraindividuel – afin de déterminer les facteurs sous-jacents à l'évolution de la durée de copulation chez ce groupe. Les durées moyennes de copulation spécifiques d'une espèce dépassent en général 25 min (Tab. I). Etant donné que le transfert du sperme et du bouchon d'accouplement (secrété par le mâle) prend moins de 10 min, la seule explication possible pour la longue durée de copulation semble être qu'elle facilite la migration du sperme dans la spermathèque. Cette hypothèse doit pourtant être testée, car certaines espèces ont des durées de copulation relativement courtes (Tab. I). Par ailleurs la durée de migration du sperme ne correspond pas entièrement au temps passé in copula.
La variation interspécifique de la durée de copulation ne peut pas s'expliquer par des différences entre espèces concernant le comportement avant l'accouplement ou la taille de l'insecte (Tab. II). Pourtant des différences entre espèces dans le comportement de protection de la femelle (en liaison avec l'existence d'accouplements multiples) et dans le taux de migration du sperme pourraient expliquer la variation interspécifique dans la durée de copulation. Au sein d'une même espèce les mâles passent un temps très variable in copula. Les derniers résultats suggèrent que les facteurs suivants contribuent tous à la variation intraspécifique : variation similaire des taux de transfert du sperme, qualité du mâle (investissement dans la physiologie de la reproduction (Tab. I), taille, immunocompétence, passé d'accouplement), réaction des mâles au statut de la femelle (par ex. vierge vs. non vierge). Finalement les mâles passent un temps variable in copula en fonction du nombre antérieur d'accouplements. Trois niveaux différents doivent être pris en compte pour expliquer la durée de copulation. Tandis que l'explication de la durée de copulation généralement longue chez *Bombus* pourrait être en rapport avec les variations de ce caractère au niveau spécifique et avec la variabilité intraspécifique, il existe vraisemblablement d'autres facteurs, par ex. la taille du mâle, pour lesquels l'explication n'est possible qu'à l'un de ces niveaux. La recherche future devrait s'attacher, par des expérimentations contrôlées, à démêler les diverses causes potentielles et les pressions de sélection qui s'exercent sur la durée de copulation des bourdons.

***Bombus* / accouplement / transfert de sperme / polyandrie / sélection sexuelle / signe de fécondation**

Zusammenfassung – Die evolutionäre Bedeutung langer Kopulationsdauern bei Hummeln. Die Kopulationsdauer – die Zeit, die ein Paar in copula verbringt – ist von zentraler Bedeutung während der sexuellen Reproduktion. Die Dauer der Kopulation wird allerdings durch eine ganze Reihe unterschiedlicher Selektionsdrücke beeinflusst, z.B. durch Kräfte der natürlichen Selektion,

die die Spermaübertragung optimieren oder die Zeit- und Energiekosten einer Kopulation minimieren. Neuere Studien zeigen jedoch, dass die Kopulationsdauer auch von sexueller Selektion beeinflusst werden kann, wobei die Männchen beispielweise ihren eigenen Reproduktionserfolg auf Kosten des weiblichen maximieren. Kopulationen bei Honigbienen (*Apis* spp.) und Stachellosen Bienen (*Meliponinae*) dauern nur einige wenige Sekunden, was darauf hinweist, dass die natürliche Selektion eine wichtige Rolle für die Evolution der Kopulationsdauer bei diesen Arten gespielt hat. Bei Hummeln der Gattung *Bombus* dauern Kopulationen wesentlich länger und variieren sowohl zwischenartlich als auch innerartlich erheblich. Dies kann als Hinweis darauf gewertet werden, dass bei Hummeln ein zwischengeschlechtlicher evolutionärer Konflikt über die ideale Länge der Kopulation vorhanden ist.

Hier präsentieren wir verfügbare Daten über das Kopulationsverhalten bei Hummeln und analysieren diese auf drei verschiedenen Ebenen: zwischenartlich, innerartlich und individuell. Dies erlaubt uns, mögliche Faktoren zu isolieren, die für die Evolution der langen Kopulationsdauer in dieser Gruppe verantwortlich sind. Die artspezifische Kopulationsdauer bei Hummeln ist typischerweise länger als 25 Minuten (Tab. I). Da der Transfer der Spermien und des Kopulationspfropfs in den weiblichen Geschlechtstrakt normalerweise weniger als 10 Minuten in Anspruch nimmt, scheint sich die lange Kopulationsdauer vor allem durch eine vereinfachte Migration der Spermien in die Spermatheka zu erklären. Diese Hypothese muss jedoch noch überprüft werden, und weitere Arbeiten sind notwendig, vor allem bei Arten mit relativ kurzer Kopulationsdauer und bei Arten, bei denen die Kopulationsdauer nicht mit der Länge der Spermienmigration in die Spermatheka übereinstimmt.

Interspezifische Unterschiede in der Kopulationsdauer können nicht durch Körpergröße oder Unterschiede im Verhalten der Geschlechter vor der *copula* erklärt werden (Tab. II). Die Unterschiede in der Kopulationsdauer zwischen unterschiedlichen Hummelarten scheint sich am besten durch die Länge des männlichen *mate guarding* (vor allem im Zusammenhang mit Polyandrie) und die Dauer der Spermienmigration in die Spermatheka erklären zu lassen. Innerartlich finden wir sehr große Unterschiede in der Zeit, die Männchen in copula mit ihren Partnerinnen verbringen. Die verfügbaren Daten zeigen, dass die innerartliche Kopulationsdauer durch eine ganze Reihe von Faktoren beeinflusst werden kann; zum Beispiel die Geschwindigkeit der Spermienmigration in die Spermatheka, die Qualität des Männchens, (gemessen als männliche Investitionen in die Reproduktionsphysiologie), die Körpergröße, die Qualität des Immunsystems, oder der reproduktive Status des Weibchens (virginell versus non – virginell). Interessanterweise wird die männliche Kopulationsdauer auch durch vorangegangene Kopulationen beeinflusst.

Die beobachtete lange Kopulationsdauer bei Hummeln muss deshalb auf drei Ebenen erklärt werden. Während Erklärungen für die generell lange Kopulationsdauer bei *Bombus*, artspezifische Unterschiede in der Kopulationsdauer wie auch innerartliche Variation in direktem Zusammenhang zueinander stehen könnten und deshalb auf allen drei Ebenen Erklärungspotenzial haben, scheinen gewisse Faktoren (wie zum Beispiel Körpergröße) nur für eine dieser Ebenen von Bedeutung zu sein. Es scheint klar, dass weitere experimentelle Arbeiten nötig sind, um die verschiedenen Faktoren und Gründe genauer zu isolieren, die die Evolution der beobachteten langen Kopulationsdauern bei Hummeln erklären können.

Paarung / Spermaübertragung / Begattungszeichen / Polyandrie / sexuelle Selektion

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