

Paternity skew in seven species of honeybees (Hymenoptera: Apidae: *Apis*)¹

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Abstract – Honeybees (*Apis*) show an extremely polyandrous mating system. In general honeybee queens mate with at least ten drones. The reproductive success of the drones is usually biased giving rise to speculations of a first or last male advantage. Especially for *A. andreniformis* and *A. florea* a first male advantage was hypothesized due to the peculiar anatomy of their male genitalia. We reanalyzed data from the literature by using a sample size calibration method to survey the differences and similarities in paternity skew among species in the genus *Apis*. The paternity skew among seven honeybee species differed significantly, particularly due to the rare patriline. The sorting algorithm, i.e. the ranking of the patrilines, had, however, a considerable effect on the paternity skew pattern. The frequent patrilines appeared to be similarly distributed in all tested species. As a consequence the proposed first male advantage in the dwarf honeybees is not supported by empirical data.

Apis / honeybee / polyandry / patrilines / paternity skew

1. INTRODUCTION

The increasing use of molecular population genetic methods have revealed in the past decade the widespread occurrence of females mating with multiple males throughout the animal kingdom (Zeh and Zeh, 2001; Knight, 2002). One of the general principles of polyandry is that males should try to maximize their semen contributions in relation to competing inseminating males. Male and sperm competition are typical for many polyandrous mating systems (Thornhill and Alcock, 1983; Simmons, 2001) resulting in male reproductive skew which is believed to be a major driving mechanism of evolution (Ross, 2001; Keller and Reeve, 1999).

Although polyandry is rare in the social Hymenoptera (bees, ants and wasps) (Strassmann, 2001), the entire genus of honeybees *Apis* L. (Hymenoptera: Apidae) is characterized by an extreme degree of polyandry. Honeybee queens typically copulate on their nuptial flights with more than ten drones (Palmer et al., 2001; Tarpy et al., 2004). In all honeybee species except the dwarf honeybees (*A. florea* Fabricius, *A. andreniformis* Smith), the drones inject the semen into the lateral oviducts of the queen. The majority of the semen is expelled by the queen and only a small fraction (ca. 10%) enters the spermatheca through Breslau's semen pump (Ruttner and Koeniger, 1971). The queen stores the semen in her spermatheca where it remains viable throughout her lifetime.

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Polyandry results in mixed paternity offspring with unequal male contributions (Estoup et al., 1994). In some species mating frequencies are extremely high. In the giant Asian honeybee, *Apis dorsata* Fabricius, females mate with more than 50 drones (Moritz et al., 1995; Oldroyd et al., 1996; Wattanachaiyingcharoen et al., 2003). Likewise, mating frequencies exceeding 40 were recently reported for the medium sized cavity nesting honeybee *A. nigrocincta* Smith (Palmer et al., 2001). In the other *Apis* species the numbers of copulations are slightly lower (Palmer and Oldroyd, 2000; Tarpy et al., 2004) but still extraordinarily high compared to bees from closely related taxa (Strassmann, 2001).

The male endophallus of the dwarf honeybees *A. andreniformis* and *A. florea* is a highly elaborate structure. It allows the drone to deposit its sperm directly into the *ductus spermaticus*, which connects the vagina with the spermatheca of the queen. As a consequence *A. florea* and *A. andreniformis* have a higher transfer efficiency with a lower percentage of expelled semen per drone: between 40 and 60% of spermatozoa per drone are retained in the queen, compared to only about 10% in the Asian cave-nesting species and less than 3% in *A. mellifera* L. (Koeniger and Koeniger, 2000). Likewise Palmer and Oldroyd (2000) estimated higher sperm transfer rates in the dwarf honeybees (*A. florea* 28%, *A. andreniformis* 74%, *A. mellifera* 4.5%, *A. dorsata* 5.5%, *A. cerana* Fabricius 7.2%, *A. koschevnikovi* v. Buttel-Reepen 7.6%). Once semen has entered the spermatheca, it cannot be easily replaced or expelled. Based on this special mode of sperm transfer in *A. andreniformis* and *A. florea*, Koeniger et al. (2000) hypothesized a reproductive advantage of the first mating males in these species. As a result one would expect striking differences in sperm usage in the dwarf honeybees as compared to the other species of the genus *Apis*. The higher semen transfer efficiency in the dwarf honeybees must result in a more pronounced paternity skew (resulting from male or semen competition) than in those honeybee species where the drones inject the semen into the lateral oviducts. In this study we review empirical data, and analyze the differences in paternity skew between the dwarf honeybees and the other honeybee species in the

genus *Apis* to reveal the potential impact of the different mating strategies.

2. MATERIALS AND METHODS

2.1. Paternity frequencies in honeybees

We reviewed data from the literature to survey the differences and similarities in the male mating success among seven honeybee species. These include all currently known honeybee species except the montane *A. nuluensis* Tingek, Koeniger and Koeniger from Borneo and the giant Himalayan honeybee *A. laboriosa* Smith. We only included paternity frequency data obtained by microsatellite DNA analyses, which allowed for particularly precise assessment of the male reproductive skew (Estoup et al., 1994). Thus, we reanalyzed studies which included the individual frequency of every detected patriline. For each species the source of the data as well as the numbers of microsatellite loci used and the numbers of investigated honeybee colonies are listed in Table I.

2.2. Measurement of paternity skew

Several reproductive skew indices were recently reviewed and compared with respect to their applicability (Tsuji and Kasuya, 2001). Though these are helpful tools, they have a drawback in that a single estimated index is used to describe an entire distribution, which may have extremely different shapes in different species and genera. Instead of using a single index we therefore employ the actual frequency distribution of the reproductive success of honeybee drones for further comparative analyses in this study.

We did not include effective paternity estimates in our study. Effective paternities are relatedness based coefficients, which are important for many evolutionary questions in insect societies (Tarpy and Nielsen, 2002; Nielsen et al., 2003). However, like reproductive skew indices, effective paternity estimates do not give information about the pattern of the frequency distribution of the patrilines.

2.3. Sample size calibration

Estimating paternity frequencies with genetic markers is prone to two types of errors. First, patrilines could remain undetected because the queen's offspring by chance may show identical genotypes despite being sired by different drones. This "non-detection-error" (Boomsma and Ratnieks, 1996) depends on the number of polymorphic alleles of the genetic markers used to analyze the population

Table I. Synopsis of the surveyed studies on the mating system in the honeybee genus *Apis*. Besides the references of the data for every species the number of analyzed DNA microsatellite loci, the number of alleles per locus, and the included number of colonies are presented. In addition the estimated numbers of matings, which can be expected to be found in a sample of 35 workers are given.

Species	Loci	Alleles per locus	Colonies	Matings	Reference
<i>Apis andreniformis</i> Smith	3–4	2–7	4	10.6	Oldroyd et al., 1997
<i>A. cerana</i> Fabricius	3–4	5–9	4	13.5	Oldroyd et al., 1998
<i>A. dorsata</i> Fabricius	3	8–21	6	19.4	Moritz et al., 1995; Oldroyd et al., 1996
<i>A. florea</i> Fabricius	5	2–5	5	12.4	Palmer and Oldroyd, 2001
<i>A. koschevnikovi</i> v. <i>Buttel-Reepen</i>	3–4	2–7	4	11.6	Rinderer et al., 1998
<i>A. mellifera</i> Linnaeus	4–8	5–27	12	16.0	Neumann et al., 1999a, b, 2000; Neumann and Moritz, 2000; Neumann, 1998; personal communication; Schlüns et al., 2005
<i>A. nigrocincta</i> Smith	5	2–7	4	22.1	Palmer et al., 2001

samples. However, the resolution of the used microsatellite loci was generally high in all studies (high numbers of loci and alleles), resulting in similar non-detection errors for the different data sets in Table I.

The second type of error is based on the sample size. Small sample sizes clearly result in missing patriline, which were not sampled. The “non-sampling-error” and sample size are therefore essential for any comparison among the data sets in Table I. The differences in the sample sizes are huge and range from 35 to the extreme of 194 genotyped workers per colony. A useful common method to determine a corrected number of patrilines from the observed ones was given by Cornuet and Aries (1980). However, their formula is based on the assumption of an equal distribution of all patrilines, which is violated in several cases in Table I. To overcome this problem, we developed a computer program (with the GNU C compiler) to standardize the sample sizes of all studies. A virtual sample of 35 workers was drawn at random from a population with patriline frequencies equivalent to the empirical sample using pseudo-random numbers. Every randomly drawn worker was assigned to its specific patriline. For each colony 100 replicates were performed and for each replicate the patrilines were sorted corresponding to their re-sampled frequencies. The sequence of patrilines could thus vary from replicate to replicate. For instance, the most frequent patriline in the original data could by chance be the second or third most frequent one in some of the re-sampling bouts. The mean frequencies for the newly assigned patrilines were determined across all 100

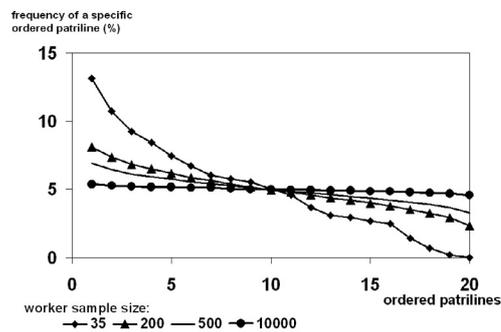


Figure 1. Frequency distribution of ordered patrilines. An equal distribution (=5%) was predetermined for 20 virtual patrilines. The biased frequency distributions result from different sample sizes of workers.

replicates. All rare patrilines (>9th) were grouped together in one class for the subsequent statistical analysis.

The relative frequencies of the patrilines in the original samples are estimates rather than the true patriline frequencies in the colonies. We therefore simulated a colony with an equal distribution of 20 patrilines in order to assess a potential bias as a result of the re-sampling method. Five different worker sample sizes (35 to 10 000; see Fig. 1) were used for this reference colony each with 100 replicates.

In order to assess the impact of a large sample size, the patriline distribution of one of the *A. dorsata* colonies (194 genotyped workers) was tested (Kolmogorov-Smirnov two sample test) against an equivalent simulated distribution starting from an equal distribution.

2.4. Statistical analysis

A correspondence analysis was performed to explore the homogeneity of the paternity skew (i.e. the relative frequencies of the patrilines ordered according to their abundance in the colony) among the seven honeybee species. The contingency table contains the average absolute frequencies of the ranked patrilines for every species. The frequencies are summed up for all colonies of each species. The figures used in the analysis correspond to counts of full-sister bees (i.e. workers of the same patriline), which can be found on the average of 100 replicates of the re-sampling method in a random sample of 35 workers. The results allow for detailed analyses of the structure of categorical variables in the contingency table (StatSoft, 2001). Here we use this method to gain information about the extent of the different contributions of the seven species (columns) and the ordered patrilines (rows) by decomposing the overall Chi-square statistic. If the rows (ranked patrilines) and columns (species) in the contingency table were completely independent of each other, the expected frequencies in the table would be equal to the respective column total times the row total, divided by the grand total. That is, the contingency table would be completely homogenous. A relative inertia is the proportion of the Chi-square of a row or column in relation to the overall Chi-square. Thus, the relative inertia of a species (or ranked patrilines, respectively) represents the contribution of this species to the deviation from the homogeneity of the contingency table. The power analysis of the Chi-square contingency test was performed with the G*Power software (Buchner et al., 1997). All other statistical tests and computations were carried out by using the STATISTICA™ software package (StatSoft, 2001). A Kruskal-Wallis test was performed to study potential differences in mating frequencies among the species.

3. RESULTS

There is a highly significant difference in the mating frequencies (=average number of patrilines) among the seven *Apis* species (Kruskal-Wallis-Test: $H = 19.86$; $DF = 6$; $n = 39$; $P < 0.005$) in the re-sampled data.

3.1. Paternity skew among and within honeybee species

The results of the re-sampling procedure are summarized in Table II. The average occurrences of the patrilines from all colonies are listed for each species. The correspondence analysis reveals a highly significant difference in the relative frequencies of the patrilines among the seven species (overall Chi-square statistic = 95.1883; $DF = 54$; $n = 1365$; $P < 0.001$). No significant differences in the paternity frequency distribution are found among colonies within species. The relative inertia weighting the contribution of a patriline or a species to the deviation from the homogeneity of the contingency table are given in Table II. The highest relative inertia is found for *Apis nigrocincta*. Likewise *A. dorsata* and *A. andreniformis* are higher than average in respect to their relative inertia. The rare patrilines contribute most to the deviation from homogeneity with a very high relative inertia (≈ 0.75). Furthermore, if the rare patrilines are omitted in the correspondence analysis, the overall Chi-square statistic drops to a very low value (= 7.22854) and the differences among the species are no longer significant. The post-hoc power analysis revealed a very low type II error ($1 - \beta = 1.0000$; type I error = 0.05; $DF = 48$; $n = 1101$; effect size = 0.3), suggesting that the nine most frequent patrilines are thus evenly distributed among the seven honeybee species. All re-sampled colonies yielded an overestimate of the most frequent patriline and an underestimate of the rarest patriline. The data therefore indicate that for small sample sizes not only the mating frequency is underestimated, but also the proportions of the patrilines are inherently biased towards the more frequent patrilines.

3.2. Simulation of a reference colony

The simulation of a colony with an equal distribution of 20 patrilines revealed a remarkable bias of the frequency distribution of the patrilines (Fig. 1). Particularly, the smallest worker sample size (=35) entailed a strong skew as a result of the re-sampling method including the re-sorting procedure of the patrilines to their rankings. Only the largest sample size (=10 000 workers) resulted in a

Table II. Paternity skew of seven *Apis* species. Average percentages of workers per patriline (\pm standard error of the means of the colonies per species) resulting from the re-sampling method and total numbers of worker for each species. The impact of the respective species on the deviation from the homogeneity of the table is presented as the relative inertia which is the sum of the Pearson Chi-square of a row or a column of a multi-way table divided by the total sum (StatSoft, 2001).

Patriline	<i>A. andreniformis</i>	<i>A. cerana</i>	<i>A. dorsata</i>	<i>A. florea</i>	<i>A. koschevnikovi</i>	<i>A. mellifera</i>	<i>A. nigrocincta</i>	<i>rel. inertia</i>
1	22.40 \pm 1.11	20.21 \pm 1.39	17.84 \pm 2.25	23.42 \pm 1.82	22.86 \pm 3.29	18.83 \pm 2.23	13.69 \pm 2.56	0.059
2	16.96 \pm 0.74	14.69 \pm 0.81	10.61 \pm 1.01	15.89 \pm 1.14	17.42 \pm 2.44	13.39 \pm 1.37	9.34 \pm 0.71	0.069
3	13.61 \pm 0.65	11.62 \pm 0.66	8.28 \pm 0.83	12.27 \pm 0.78	13.77 \pm 1.73	10.29 \pm 0.93	7.58 \pm 0.45	0.056
4	11.16 \pm 0.50	9.61 \pm 0.51	7.10 \pm 0.50	9.78 \pm 0.40	10.61 \pm 0.10	8.37 \pm 0.56	6.71 \pm 0.29	0.033
5	9.19 \pm 0.37	8.06 \pm 0.33	6.30 \pm 0.32	8.00 \pm 0.19	8.33 \pm 0.55	7.07 \pm 0.37	5.93 \pm 0.15	0.018
6	7.46 \pm 0.29	6.72 \pm 0.23	5.70 \pm 0.29	6.51 \pm 0.20	6.29 \pm 0.43	6.46 \pm 0.31	5.54 \pm 0.14	0.006
7	5.90 \pm 0.33	5.76 \pm 0.06	5.17 \pm 0.31	5.41 \pm 0.22	4.99 \pm 0.57	5.76 \pm 0.04	5.10 \pm 0.17	0.003
8	4.53 \pm 0.44	5.14 \pm 0.14	4.31 \pm 0.42	4.50 \pm 0.25	3.91 \pm 0.70	4.63 \pm 0.38	4.52 \pm 0.22	0.003
9	3.32 \pm 0.49	4.41 \pm 0.24	3.90 \pm 0.36	3.74 \pm 0.29	2.91 \pm 0.79	4.21 \pm 0.36	3.71 \pm 0.22	0.007
>=10	5.46 \pm 2.58	13.79 \pm 3.32	30.79 \pm 4.84	10.47 \pm 3.09	8.91 \pm 7.32	21.00 \pm 3.75	37.88 \pm 4.45	0.746
Total	140	140	210	175	140	420	140	1.000
rel. inertia	0.193	0.030	0.196	0.100	0.128	0.013	0.338	

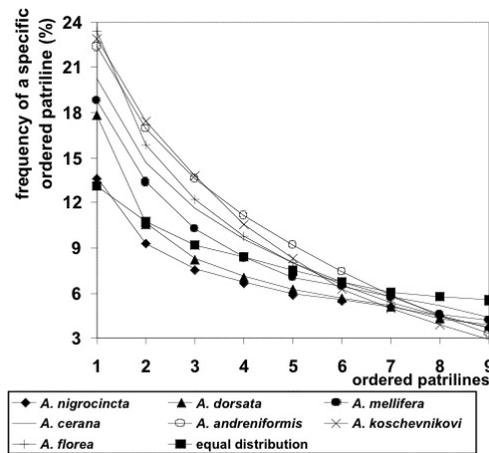


Figure 2. Observed and expected patriline frequencies under equal sperm contribution in a sample of 35 workers: Kolomogorov-Smirnov two sample tests revealed for all comparisons non-significant differences ($P > 0.01$) between the equal distribution (conf. Fig. 1) and each of the empirical, re-sampled distributions (*A. nigrocincta*: $l_{d_{\max}}$ = 0.44; *A. dorsata*: $l_{d_{\max}}$ = 0.33; *A. mellifera*: $l_{d_{\max}}$ = 0.22; *A. cerana*: $l_{d_{\max}}$ = 0.22; *A. andreniformis*: $l_{d_{\max}}$ = 0.33; *A. koschevnikovi*: $l_{d_{\max}}$ = 0.33; *A. florea*: $l_{d_{\max}}$ = 0.33).

distribution that resembled approximately the true distribution. Thus paternity skews are inevitable just due to the sampling variance alone.

3.3. Empirical skew versus re-sampled equal distribution

A comparison of the re-sampled empirical data with the simulated data based on equal patriline distribution shows no significant differences in each of the comparisons (Fig. 2). The Kolomogorov-Smirnov two sample test shows that there is a significant difference ($l_{d_{\max}}$ = 0.43; $P < 0.01$) between the patriline distribution of the *A. dorsata* colony with 194 genotyped workers and an equivalent simulated distribution starting from an equal distribution.

4. DISCUSSION

4.1. Number of matings and paternity skew

Using our sample size calibration method, we could confirm Palmer et al.'s (2001) notion

that *A. nigrocincta* shows the highest average mating frequency of all currently studied *Apis* species (Tab. I). The data for *A. dorsata* are in agreement with the review of Palmer and Oldroyd (2000). The estimate for the western honeybee *A. mellifera* (16.0) is in good concordance with the classical population genetic study by Adams et al. (1977), which yielded an average mating frequency of 17.25 with a maximum likelihood estimate based on the frequency of diploid males. Similarly, Neumann and Moritz (2000) obtained a mean mating number of 17.7 in 89 *A. mellifera* colonies by genotyping 1290 workers with DNA microsatellites.

The estimates for the degree of polyandry thus appear to be robust, in spite of the large variance in sample size and methods used to determine mating frequencies. We can confirm the extreme degree of polyandry in all seven *Apis* species (Palmer and Oldroyd, 2000). We found still significantly different mating frequencies among the species after applying the sample size calibration method despite of relatively small sample sizes. This finding is consistent with hitherto reported significant differences in mating frequencies in the genus *Apis* (Tarpy et al., 2004).

4.2. Sampling bias of patriline frequencies

The most surprising result is the homogeneity of the frequency distribution of the nine most frequent patrilines. In spite of the significantly different mating frequencies, and in spite of the extremely different mode of semen transfer in the dwarf honeybees, we obtain an astonishing similarity among the seven *Apis* species tested. Moreover, we obtain this result even though the species differ substantially in numbers of spermatozoa (from 0.14 to more than 10 million; Koeniger and Koeniger, 2000; Palmer and Oldroyd, 2000).

The uniform pattern in paternity skew of the most frequent patrilines in honeybees stems at least partly from the sorting algorithm. The small sample sizes along with the ranking of the patrilines (based on the obtained frequencies) result in biased skews. This mechanism equally affects all species. As we can see from our resampling results, the obtained ranking orders of the patrilines always differ from the original

ranking in that the high-ranking patriline are over-estimated in their frequencies whereas rare patriline are under-estimated or they even get lost completely. The small sample sizes that were used in many studies do not allow us to discriminate between an equal distribution and the seemingly "skewed" patriline frequencies in the empirical data. Nevertheless, we could find a statistically significant deviation from an equal distribution, when we applied our method to a large sample size in one of the *A. dorsata* colonies (194 workers). The differences in the rare patriline seem to result from the differences in mating frequencies among the species and hence the varying total number of patriline.

4.3. First male or last male advantage?

The results of the current study do not support the notion of a different mating system in the dwarf honeybees (first male advantage) and the other honeybee species. We found no differences in the paternity skew between the dwarf honeybees and all other species in the frequent patriline. The dwarf honeybees are characterized by highly elaborate male genitalia. Although the injection into the spermatheca seems to affect the percentage of spermatozoa per drone reaching the spermatheca, this appears to have little effect on the paternity skew. This is clearly surprising because we would have expected very different dynamics in semen handling between the dwarf honeybees (semen is injected into the spermathecal duct) and the other species (semen is injected into the lateral oviducts). Sperm clumping as a cause for the pattern of the paternity skew is unlikely. It only rarely occurs in honeybees (if at all) and despite one contrary claim (Taber, 1955), semen clumping was not found in a series of studies on instrumentally and naturally mated *A. mellifera* queens (Moritz, 1983; Laidlaw and Page, 1984; Haberl and Tautz, 1998; Schlüns et al., 2004). A decrease of the variance of subfamily frequencies was reported twice (Franck et al., 1999, 2002) suggesting that the degree of sperm mixing within the spermatheca increases with time. Moreover, the semen volumes produced by the drones have a significant impact on the patriline frequencies, whereas the rank in the sequence of copulations don't (Franck et al., 2002; Schlüns et al., 2004).

We cannot exclude that there is a slight advantage of the first drones copulating with the queen in the dwarf honeybee species. However, even if there were such an effect, it was not detectable using our re-sampling method and the data from the literature with the usual sample sizes. Thus, we have no statistical support for a major difference between the spermathecal-duct-injecting species and the oviduct-injecting species.

The sample size effect and the sorting procedure effect are not only important for frequency distributions of patriline in honeybee colonies. The same problem occurs in polygynous ant colonies, where the number of matriline can be substantially skewed (Fraser et al., 2000). Likewise, male offspring in bumble bee nests can be produced by several females (Lopez-Vaamonde et al., 2004). Here, the same problems can arise if sample sizes are small and the true ranking of the individuals with respect to the reproductive dominance is not known in advance or independently established.

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Résumé – Asymétrie d'apparement génétique chez sept espèces d'abeilles mellifères (Hymenoptera : Apidae : Apis). Les abeilles mellifères (du genre *Apis* L.) présentent un système d'accouplement extrêmement polyandre : en général les reines s'accouplent avec au moins dix mâles, mais plus de 50 accouplements ont été détectés chez deux espèces, *A. dorsata* Fabricius et *A. nigrocincta* Smith. Le succès reproductif des mâles qui produisent des descendants est en général variable, ce qui a conduit à des spéculations sur l'avantage au premier ou au dernier mâle. On a émis l'hypothèse de l'avantage au premier mâle en particulier pour les abeilles naines (*A. andreniformis* Smith et *A. florea* Fabricius) en raison de l'anatomie particulière de leurs organes de reproduction mâles, qui permet d'injecter le sperme directement dans le canal spermatique. Afin de passer en revue les différences et les similitudes dans l'asymétrie d'apparement parmi les espèces du genre *Apis*, nous avons ré-analysé les données de la littérature en

utilisant une méthode d'étalonnage de la taille de l'échantillon. Il en résulte que l'asymétrie d'apparement diffère grandement entre les sept espèces, particulièrement en raison de lignées paternelles rares. L'algorithme de tri, i.e. le classement des lignées paternelles a pourtant une influence énorme sur le profil d'asymétrie de l'apparement. Les lignées paternelles fréquentes semblent être réparties de la même façon dans toutes les espèces testées, suggérant un mécanisme commun de transfert du sperme pour toutes les abeilles mellifères. En conséquence l'avantage au premier mâle proposé pour les abeilles naines ne semble pas être corroboré par les données empiriques.

***Apis* / polyandrie / lignée paternelle / asymétrie d'apparement**

Zusammenfassung – Paternity skew bei sieben Honigbienenarten (Hymenoptera: Apidae: Apis). Honigbienen (*Apis* L.) weisen ein extrem polyandrisches Paarungssystem auf. Im Allgemeinen paaren sich Honigbienenköniginnen mit mindestens zehn Drohnen, aber es wurden bei zwei Arten (*A. dorsata* Fabricius und *A. nigrocincta* Smith) auch mehr als 50 Paarungen festgestellt. Der Fortpflanzungserfolg der Nachkommen zeugenden Drohnen ist üblicherweise sehr unterschiedlich, was zu Spekulationen über einen Vorteil des zuerst- oder zuletztbegattenden Männchens geführt hat. Insbesondere für die Zwerghonigbienen (*A. andreniformis* Smith und *A. florea* Fabricius) wurde die Hypothese eines Vorteils des zuerstbegattenden Männchens aufgrund der eigentümlichen Anatomie ihrer männlichen Genitalien, die eine direkte Injektion des Spermas in den Spermadukt erlauben soll, aufgestellt. Um Unterschiede und Ähnlichkeiten im „paternity skew“ zwischen Arten der Gattung *Apis* zu untersuchen, haben wir Daten aus der Literatur mittels einer Kalibrierungsmethode für Stichprobengrößen reanalysiert. Im Ergebnis unterscheiden sich die sieben Honigbienenarten im „paternity skew“ signifikant, vor allem aufgrund der seltenen Patrilinearität. Der Sortierungsalgorithmus, d.h. die Rangfolge der Patrilinearität, hatte jedoch einen beträchtlichen Effekt auf das Muster des „paternity skews“. Die häufiger auftretenden Patrilinearitäten scheinen bei allen untersuchten Arten ähnlich verteilt zu sein. Dies lässt auf einen allgemeinen Spermienübertragungsmechanismus für alle Honigbienen schließen. Folglich wird der vermutete Vorteil der zuerstbegattenden Männchen bei den Zwerghonigbienen nicht durch die empirischen Daten unterstützt.

***Apis* / Honigbiene / Polyandrie / Patrilinearität / paternity skew**

REFERENCES

- Adams J., Rothman E.D., Kerr W.E., Paulino Z.L. (1977) Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*, *Genetics* 86, 583–596.
- Boomsma J.J., Ratnieks F.L.W. (1996) Paternity in eusocial Hymenoptera, *Phil. Trans. R. Soc. Lond. B* 351, 947–975.
- Buchner A., Erdfelder E., Faul F. (1997) How to Use G*Power. http://www.psych.uni-duesseldorf.de/aap/projects/gpower/how_to_use_gpower.html (accessed on 10 February 2005).
- Cornuet J.-M., Aries F. (1980) Number of sex alleles in a sample of honeybee colonies, *Apidologie* 11, 87–93.
- Estoup A., Solignac M., Cornuet J.-M. (1994) Precise assessment of number of patriline and of genetic relatedness in honeybee colonies, *Proc. R. Soc. Lond. B* 258, 1–7.
- Franck P., Coussy H., Le Conte Y., Solignac M., Garnery L., Cornuet J.-M. (1999) Microsatellite analysis of sperm admixture in honeybee, *Insect Mol. Biol.* 8, 419–421.
- Franck P., Solignac M., Vautrin D., Cornuet J.-M., Koeniger G., Koeniger N. (2002) Sperm competition and last-male precedence in the honeybee, *Anim. Behav.* 64, 503–509.
- Fraser V.S., Kaufmann B., Oldroyd B.P., Crozier R.H. (2000) Genetic influence on caste in the ant *Camponotus consobrinus*, *Behav. Ecol. Sociobiol.* 47, 188–194.
- Haberl M., Tautz D. (1998) Sperm usage in honey bees, *Behav. Ecol. Sociobiol.* 42, 247–255.
- Keller L., Reeve H.K. (1999) Dynamics of conflicts within insect societies, in: Keller L. (Ed.), *Levels of selection in Evolution*, Princeton University Press, pp. 153–175.
- Knight J. (2002) Sexual stereotypes, *Nature* 415, 254–256.
- Koeniger N., Koeniger G. (2000) Reproductive isolation among species of the genus *Apis*, *Apidologie* 31, 313–339.
- Koeniger G., Koeniger N., Tingek S., Kelitu A. (2000) Mating flights and sperm transfer in the dwarf honeybee *Apis andreniformis* (Smith, 1858), *Apidologie* 31, 301–311.
- Laidlaw H.H. Jr., Page R.E. Jr., (1984) Polyandry in honey bees (*Apis mellifera* L.): sperm utilization and intracolony genetic relationships, *Genetics* 108, 985–997.
- Lopez-Vaamonde C., Koenig J.W., Brown R.M., Jordan W.C., Bourke A.F.G. (2004) Social parasitism by male-producing reproductive workers in a eusocial insect, *Nature* 430, 557–560.
- Moritz R.F.A. (1983) Homogenous mixing of honeybee semen by centrifugation, *J. Apic. Res.* 22, 249–255.
- Moritz R.F.A., Kryger P., Koeniger G., Koeniger N., Estoup A., Tingek S. (1995) High degree of

- polyandry in *Apis dorsata* queens detected by DNA microsatellite variability, *Behav. Ecol. Sociobiol.* 37, 357–363.
- Neumann P. (1998) The impact of polyandry and drifting on the genotypic composition of honeybee (*Apis mellifera* L.) colonies, PhD Thesis, Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany.
- Neumann P., Moritz R.F.A. (2000) Testing genetic variance hypotheses for the evolution of polyandry in the honeybee (*Apis mellifera* L.), *Insectes Soc.* 47, 271–279.
- Neumann P., Moritz R.F.A., Mautz D. (1999a) Using DNA microsatellites for maternity testing in honeybees (*Apis mellifera* L.), *Apidologie* 30, 505–512.
- Neumann P., Moritz R.F.A., van Praagh J.P. (1999b) Queen mating frequency in different types of honey bee mating apiaries, *J. Apic. Res.* 38, 11–18.
- Neumann P., Moritz R.F.A., Mautz D. (2000) Colony evaluation is not affected by drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary, *Apidologie* 31, 67–79.
- Nielsen R., Tarpy D.R., Reeve H.K. (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population, *Mol. Ecol.* 12, 3157–3164.
- Oldroyd B.P., Smolenski A.J., Cornuet J.-M., Wongsiri S., Estoup A., Rinderer T.E., Crozier R.H. (1996) Levels of polyandry and intracolony genetic relationships in *Apis dorsata* (Hymenoptera: Apidae), *Ann. Entomol. Soc. Am.* 89, 276–283.
- Oldroyd B.P., Clifton M.J., Wongsiri S., Rinderer T.E., Sylvester H.A., Crozier R.H. (1997) Polyandry in the genus *Apis*, particularly *Apis andreniformis*, *Behav. Ecol. Sociobiol.* 40, 17–26.
- Oldroyd B.P., Clifton M.J., Parker K., Wongsiri S., Rinderer T.E., Crozier R.H. (1998) Evolution of mating behavior in the genus *Apis* and an estimate of mating frequency in *Apis cerana* (Hymenoptera: Apidae), *Ann. Entomol. Soc. Am.* 91, 700–709.
- Palmer K.A., Oldroyd B.P. (2000) Evolution of multiple mating in the genus *Apis*, *Apidologie* 31, 235–248.
- Palmer K.A., Oldroyd B.P. (2001) Mating frequency in *Apis florea* revisited (Hymenoptera, Apidae), *Insectes Soc.* 48, 40–43.
- Palmer K.A., Oldroyd B.P., Franck P., Hadisoelilo S. (2001) Very high paternity frequency in *Apis nigrocincta*, *Insectes Soc.* 48, 327–332.
- Rinderer T.E., Stelzer J.A., Oldroyd B.P., Tingek S. (1998) Levels of polyandry and intracolony genetic relationships in *Apis koschevnikovi*, *J. Apic. Res.* 37, 281–287.
- Ross K.G. (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure, *Mol. Ecol.* 10, 265–284.
- Ruttner F., Koeniger G. (1971) Die Füllung der Spermatheka der Bienenkönigin, *Z. Vgl. Physiol.* 72, 411–422.
- Schlüns H., Koeniger G., Koeniger N., Moritz R.F.A. (2004) Sperm utilization pattern in the honeybee (*Apis mellifera*), *Behav. Ecol. Sociobiol.* 56, 458–463.
- Schlüns H., Moritz R.F.A., Neumann P., Kryger P., Koeniger G. (2005) Multiple nuptial flights, sperm transfer, and the evolution of extreme polyandry in honeybee queens, *Anim. Behav.* (in press).
- Simmons L.W. (2001) Sperm competition and its evolutionary consequences in the insects, Princeton University Press, Princeton.
- StatSoft I. (2001) STATISTICA für Windows, StatSoft, Inc., Tulsa, OK.
- Strassmann J.E. (2001) The rarity of multiple mating by females in the social Hymenoptera, *Insectes Soc.* 48, 1–13.
- Taber S.I. (1955) Sperm distribution in the spermatheca of multiple-mated queen honey bees, *J. Econ. Entomol.* 48, 522–525.
- Tarpy D.R., Nielsen D.I. (2002) Sampling error, effective paternity, and estimating the genetic structure of honey bee colonies (Hymenoptera: Apidae), *Ann. Entomol. Soc. Am.* 95, 513–528.
- Tarpy D.R., Nielsen R., Nielsen D.I. (2004) A scientific note on the revised estimates of effective paternity frequency in *Apis*, *Insectes Soc.* 51, 203–204.
- Thornhill R., Alcock J. (1983) The evolution of insect mating systems, Harvard University Press, Cambridge, MA, USA.
- Tsuji K., Kasuya E. (2001) What do the indices of reproductive skew measure? *Am. Nat.* 158, 155–165.
- Wattanachaiyingcharoen W., Oldroyd B.P., Wongsiri S., Palmer K., Paar J. (2003) A scientific note on the mating frequency of *Apis dorsata*, *Apidologie* 34, 85–86.
- Zeh J.A., Zeh D.W. (2001) Reproductive mode and the genetic benefits of polyandry, *Anim. Behav.* 61, 1051–1063.