THE IMPACT OF POLYANDRY AND DRIFTING ON THE GENOTYPIC COMPOSITION OF HONEYBEE (*APIS MELLIFERA* L.) COLONIES

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I PETER NEUMANN, JOB P. VAN PRAAGH, ROBIN F. A. MORITZ, JOST H. DUSTMANN (1998) Testing the reliability of the potential honeybee mating area Baltrum using virgin queens and single-locus DNA-fingerprinting. Submitted to *Apidologie*

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III PETER NEUMANN, KIM FONDRK, ROBERT E PAGE Jr., ROBIN FA MORITZ (1997) Testing the reliability of DNA microsatellites in instrumentally inseminated queen honeybees (*Apis mellifera* L.). (Zuverlässigkeitstest für DNA-Microsatelliten in instrumentell begatteten Bienenköniginnen [*Apis mellifera* L.]). In: Crailsheim K, Stabenhteiner, A (eds.) *Soziale Insekten, IUSSI-Tagung Graz 1997*, Graz, Austria, p 66, ISBN 3-901864-00-8

IV PETER NEUMANN, ROBIN FA MORITZ, DIETER MAUTZ (1997) Testing the reliability of the honeybee performance yard Schwarzenau using DNA microsatellite variability. *Apidologie* 28: 216-217

V PETER NEUMANN, ROBIN FA MORITZ (1998) The impact of polyandry on the phenotype of honeybee (*Apis mellifera* L.) colonies. Submitted to *Behavioral Ecology*

VI PETER NEUMANN, ROBIN FA MORITZ (1998) Nestmate recognition and the impact of foreign bees on colonial phenotypes of *Apis mellifera*. Manuscript

INTRODUCTION

The evolution of altruistic behaviour has been one of the most puzzling and disputed questions of evolutionary biology. Darwin himself recognised that it was difficult to plausibly explain the presence of sterile individuals in social insect colonies with his theory of natural selection (Darwin 1859). Hamiltonian kin selection theory (Hamilton 1964a,b) offered a genetical solution to Darwin's dilemma. Individuals can improve their fitness not only by reproducing themselves, but also by helping other individuals carrying the same genes to reproduce. Thus, evolutionary success can be measured by inclusive instead of individual fitness (Hamilton 1964a,b).

Particularly strong selective advantages have been assumed for societies in which individuals are closely related (Hamilton 1964a,b). As a result of the haplo diploid mode of determining sex, the species of the order Hymenoptera seem to be prone to evolve altruistic behaviour. Indeed, it seems as if eusociality has been evolved at least twelve times among the Hymenoptera (Crozier & Pamilo 1996) whereas it has been only evolved once in the order Isoptera. In the simplest case a singly inseminated queen is the mother of all the female and male offspring in the colony. In such a Hymenopteran society relatedness among female offspring is 0.75. With few examples (Moritz & Southwick 1992) males usually arise from unfertilised eggs, genetically resembling gametes of the queen. Thus, relatedness among male offspring is 0.5.

However, in many species of social insects females mate more than once (Crozier & Pamilo 1996, Boomsma & Ratnieks 1996). This seems particularly counterintuitive in light of inclusive fitness benefits derived from monandry (Hamilton 1964a,b, Gadagkar 1990) and in light of potential costs of polyandry (Moritz *et al* 1995, Oldroyd *et al* 1996, 1997 but see Ratnieks 1990). Due to the three successive stages of the mating process between males and females (Page 1986), reliable documented paternity is restricted to three isolated, highly eusocial taxa, *Atta* leaf cutting ants, wasps of the genus *Vespula* and honeybees (Boomsma & Ratnieks (1996). These authors argued that multiple mating, by lowering the relatedness between female offspring and thereby the benefits of reproductive helping behavior, has not been a general constraint for the evolution of eusociality in the Hymenoptera. But, in many species of eusocial wasps (Itô 1987) and ants (Hölldobler & Wilson 1990) a low relatedness is achieved by polygyny, the presence of more than one egglaying queen in one colony. This clearly shows that low levels of intracolonial relatedness are a common feature among eusocial insects.

Consequently, the evolution of polyandry and/or polygyny is one of the central questions of evolutionary biology (Crozier & Page 1980, Ratnieks & Boomsma 1995). Most theoretical and empirical studies focused on the genetic variance hypotheses (Crozier & Page 1985, Pamilo 1993, Keller & Reeve 1994) which assume that colonies gain fitness through a low intracolonial relatedness. Several potential mechanisms have been proposed to explain such an increase in colony fitness (Page 1980, Crozier & Page 1985, Sherman *et al* 1988, Ratnieks 1990). But the impact of worker genotypic diversity on the level of the colonial phenotype remains unclear.

In light of the predicted fitness advantages derived from close relatedness, social insect colonies should be societies which carefully scrutinize nest mates and non nest mates. Indeed, good evidence for colony recognition has been presented in many species (Crozier & Pamilo 1996). The environment can substantially contribute to the colony odor (Stuart 1987, 1988), but it has been shown that also genetic determinants are highly important (reviewed by Waldmann *et al* 1988).

SOCIAL CHARACTERISTICS OF HONEYBEES, *APIS MELLIFERA*

A honeybee colony typically consists of a single egglaying long-lived queen, anywhere from zero to several thousand drones (depending on the season) and usually 10000 to 60000 workers (Moritz & Southwick 1992). Virgin queens leave their hives for 1 to 5 nuptial flights (Alber *et al* 1955, Roberts 1944), have been shown to mate at a drone congregation area and return to the colony (Ruttner and Ruttner 1966, 1972). The maximum observed mating flight range was 16 km in mainland Canada (Peer 1957). During their flights the queen usually has the possibility to mate with many drones from different colonies and apiaries (Ruttner and Ruttner 1966, 1972). The mating behaviour of a queen honey bee profoundly affects the genotypic composition of the intracolony worker population. Honeybee colonies usually consist of several subfamilies, patrilines (Adams *et al* 1977, Estoup *et al* 1994, I, II, V) because of the mixing of sperm from different males within the spermatheca of the queen (Page 1982). Members of the same subfamily are called "super sisters" (Moritz & Southwick 1992). They share both a mother queen and a drone father, and have an average 75% of their genes in common by descent. Members of different subfamilies, have been called "half-sisters" (Moritz & Southwick 1992), have different drone fathers and share only 25% of their genes. The average intracolonial relatedness among progeny honeybee worker nestmates seems to be low. Estimates range from close to 0.25 (Laidlaw & Page 1984) to 0.28 (IV). The estimated degree of polyandry in honeybees ranges from 1 to 37 according to different authors and methods of investigation (Taber 1954, Triasko 1956, Peer 1956, Woyke 1960, Gary 1963, Adams *et al* 1977, Estoup *et al* 1994, I, II, V). So far, estimates range up to an average of 17.25 effective matings (Adams *et al* 1977). It seems as if there are distinct ecological determinants of honeybee mating behavior (Koeniger 1991) such as the place and height of mating, the conditions of the drone congregation area, the drone density and the weather or climate (Meinen 1970, Englert 1972, Verbeek 1976). But, little is known about the impact of these factors on the number of times queens mate.

In contrast, the mating behaviour of the queen does not effect the genotypic composition of the intracolonial drone population. In haplo-diploid Hymenoptera drones have, with few exceptions due to homozygosity at the sex determining locus, no father and resemble gametes of the queen. Thus, regardless of the level of polyandry of the queen, the relatedness of workers to the queen's sons should be 0.25.

However, queens are not the only colony members capable of reproduction. Honeybee workers cannot mate but can lay male eggs. But, male production by workers in the honey bee is rare due to worker policing (Ratnieks 1988). Honey bee workers cannibalise eat eggs laid by other workers (Ratnieks & Visscher 1989) because queen-laid and worker-laid eggs are

recognised by means of a queen-produced egg-marking pheromone (Ratnieks 1995). Page and Erickson (1988) found rare cases of worker reproduction in queenright colonies, and using genetic variation in body colour Visscher (1989) showed that approximately one male in a thousand reared in queenright colonies are sons of workers. A rare case of an "anarchic" colony with many worker derived drones has been described (Oldroyd *et al*. 1994).
In the subspecie *A. mell. capensis* laying workers produce parthenogenetically females thorough thelytoky, instead of males (Onions 1912). Consequently, inclusive fitness theory predicts the absence of worker policing in queenright colonies of that subspecie (Greeff 1996b).

It is well established that in spite of the guard bees which scrutinize incoming bees and reject non-nest members (Butler & Free 1952, Breed & Julian 1992), drones and workers from foreign colonies can enter the hive and are adopted as new nest members (Rauschmayer 1928, Butler 1939). Accorti (1991) proposed a hypothesis that honeybees have a marked tendency to "wander" among colonies. This drifting behavior of drones and workers is a result of individual orientation errors (Rauschmayer 1928, Butler 1939) and a variety of proximate factors which have been studied in some detail (Free 1958, Free and Spencer Booth 1961, Jay 1969a,b 1971, Vollbehr 1975, Currie 1982, Currie 1986, Currie & Jay 1988, Kühl & Neumann 1996, Moritz & Neumann 1996). In spite of several predictions and assumptions addressing the potential effects of drifting on the phenotype of honeybee colonies (Lecômte 1958, Jay 1968, Poltev 1968, Di Jong *et al* 1982, Matheson 1984, Currie 1987 among others), little is known about the actual impact of drifting on the phenotype of host colonies.

There are several additional mechanisms which can potentially influence the genotypic composition of the intracolonial worker population.

1) Swarming: Although it has recently been shown that kin recognition has only a minor impact for the distribution of the patrilines (< 2%, Kryger 1997) swarming can be important. The genotypic composition may be altered through different propensities for swarming in the various subfamilies (Kryger 1997). This may profoundly affects the genotypic composition of colonies during the swarming season.

2) Differential survival of patrilines: Nothing is known about a potential different winter survival of patrilines. Likewise a hypothetical impact due to a different susceptibility of patrilines to parasites and pathogens may play a role.

3) Supersedure: The supersedure of queens can lead to the rare coexistence of two matrilineal lineages in one honeybee colony (Cooper 1986). Some races of honeybees seem to be more likely to supersede than others (Cooper 1986).

4) Merging of colonies: Another mechanism might be the merging of honeybee colonies which can follow absconding events (Hepburn *et al*, submitted) or the artificial swarm technique used by the beekeepers (Moritz 1988, Moritz & Southwick 1992).

However, most of the above mentioned phenomena do affect the genotypic composition of the colony only during the limited time window of a worker cohort's life span. Therefore, the major factors influencing the genotypic composition of honeybee colonies over a long time scale are the polyandry of the queen and the drifting of worker and drone individuals.

Selective honeybee breeding

As a result of the complex honeybee mating system one of the central questions of selected honeybee breeding certainly is: Who mates with whom? The DNA microsatellite technology offers a possibility to answer that question in a way superior to all methods used so far. In particular, potential experimental artefacts due to marker phenotypes can be avoided.

It is possible to genotype worker offspring or pooled drone pupae samples to verify honeybee breeding lines (IV) This allows to test the reliability of institutions involved in commercial honeybee breeding such as mating apiaries (I, II) and performance yards (IV, VI). This method can be used to evaluate the isolation of a mating apiary (I) and to assess mating success on that stations (II). So far, mating success has been evaluated through the number of successfully mated queens which seems to be only a rough measure in light of the high degree of polyandry in honeybees.

Evaluating performance data of honeybee sister queen colonies can suffer from two short comings caused by undesired group composition:
1. The tested "Sister queen" groups are not composed of sister queens due to mistakes in queen rearing management.
2. The drifting of drones and workers can profoundly effect the composition of the tested colonies (IV, VI).
Drifting can interfere with evaluating performance data as a result because of a loss/gain of foragers for colonies due to relevant worker population shifts. This is strongly determined by the apiary layout (Jay 1969, VI). Another impact factor might be the transmission of various pathogens and parasites as assumed by various authors (Poltev 1968).
DNA microsatellites can be used to detect these unrelated queens, workers or drones (IV, V). A combination of the drift data with the colonial phenotype data allows to quantify the impact of drifting.

CONCLUSIONS

In this thesis a range of 1 (I) to 28 (II, V) observed paternities was found for colonies with naturally mated honeybee (*Apis mellifera* L.) queens. This clearly shows the high variance for queen mating frequency in this specie. The average intracolonial relatedness among worker nestmates found in this study is 0.28 (V).

My results indicate that successful mating flights are possible on a drone-free island (I) but the significantly lower mating frequency compared to the neighbouring islands with drone colonies, suggests that mating conditions were difficult (I). My comparisons of queen mating frequency show:
1. There are distinct ecological determinants of polyandry (II). Queens mated on islands showed significant lower mating frequencies compared to queens which were mated on the

mainland (II). I conclude that distinct climatic conditions between island and the mainland are the most parsimonious explanation for the lower mating frequency of isle-mated queens (II).
2. Large distances over open water in combination with a lack of drone producing colonies, can reduce mating-frequency (I).
The high variance and the ecological determinants indicates that the level of polyandry of queens may be population specific in honeybees.

The impact of polyandry on the level of the colonial phenotype was weak and in no case significant (V). However, I found non significant trends that polyandry positively effects colony productivity (V) as predicted by the hypotheses for the evolution of polyandry.

Since the drifting of drones and workers was simultaneously studied I could evaluate and compare the levels of emigrating and immigrating workers and drones. Drones emigrate and immigrate significant more frequently than workers (V). The immigration and emigration of drones was significantly correlated but not of workers (V). Moreover, there were no significant correlations between immigration or emigration of workers and drones (V). This indicates that the adoption/rejection mechanism by the guard bees is different between drones and workers (V). There was no correlation between the number of effective queen matings and the immigration and emigration of workers and drones. Therefore I could reject the foreign label rejection model for nestmate recognition (VI). But, I could not distinguish between the other models (VI). I found no significant impact of the drifting of drones and workers on the phenotype of honeybee colonies (VI). This indicates that drifting of drones and workers is not a particular cause for colony levels of infestation with the ectoparasitic mite *Varroa jacobsoni* (VI). Likewise, I conclude that drifting has no or only weak effects on colony honey yields, as long as the drifting of workers is reduced due to apiary layout (VI).

Selective honey bee breeding

For the first time, DNA-fingerprinting was used in this thesis to test the reliability of procedures involved in selective honeybee breeding.

A reliability test for the potential honeybee mating area Baltrum was performed (I). The test shows that controlled matings on island mating yards laying within the maximum combined mating flight range of queens and drones can not be guaranteed (I). Successful mating flights of queens over open water have been found although the next available source of sexual mature drones was at least 5.4 km away (I). Matings between the Baltrum queens and drones from the mainland have been found (I). However, interactions with the neighbouring established mating areas Langeoog and Norderney were unlikely (I).

The mating success on the island mating yards Langeoog, Norderney, on the low land yards Gramschatzer-Wald and Königswald, on the highland mating yard Rachel-Diensthütte and on the isolated high mountain station Hochgrat. was assessed (II). A lower mating success was found on the two island mating apiaries compared to the other mating yards (II). The number of drone colonies at a mating yard showed no significant effect on queen mating frequency (II). Thus, most likely unfavorable weather conditions on the islands were responsible for the low mating success (II).

The reliability of the performance yard Schwarzenau was tested (IV, VI). Drifting of workers was distinctly lower in Schwarzenau than in previously studies of commercial apiaries (IV, VI). Drifting has no significant influence on evaluating performance data at Schwarzenau (IV, VI). However, unrelated queens could seriously distort results because one of the tested breeding lines was identified to consist of unrelated queens (IV). This indicates that mistakes in queen rearing management have been taken place (IV).

Reliability test for honeybee DNA microsatellites

The reliability of the employed DNA microsatellite technique (Estoup *et al* 1993, 1994) was tested using a queen which was instrumentally inseminated with the semen of 10 drones (III). In a double blind test 10 patrilines and no visible mutational events were found indicating that this technique used for honeybees is as reliable as it is in forensic medicine (III).

REFERENCES

Accorti M (1991) Indagine preliminare sulla deriva delle api. Apicoltura 7, 1-15

Adams J., Rothmann 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

Alber M, Jordan R, Ruttner F, Ruttner H (1955) Von der Paarung der Honigbiene, Z Bienenforsch 3, 1-28

Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. Phil Trans R Soc B 331, 947-975

Breed MD, Julian GE (1992) Do simple rules apply in honey-bee nestmate recognition? Nature 357: 685-686

Butler C.G. (1939) The drifting of drones. Bee World 20:140-142

Butler C.G., Free JB (1952) The behaviour of worker honeybees at the hive entrance. Behaviour 4, 262-292

Cooper BA (1986) The honeybees of the British isles. Bee Improvement and Bee Breeding Association, Derby, UK

Crozier RH, Page RE Jr. (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. Behav Ecol Sociobiol 18: 105-115

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford UK

Currie RW (1987) The biology and behaviour of drones. Bee world 68, 129-143

Currie RW and Jay SC (1988) The influence of a colony's queen state, time of the year, and drifting behaviour, on the acceptance and longevity of adult drone honeybees (*Apis mellifera* L.). Journal of Apicultural Research 27 (4): 219-226

Currie RW and Jay SC (1991) The influence of a colony's queen state on the drifting of drone honey bees (*Apis mellifera* L). Apidol 22: 183-195

Darwin C (1859) On the origin of species. Watts & Co, London

Estoup A, Solignac M, Harry M, Cornuet JM (1993) Characterisation of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. Nuc Acid Res 21, 1427-1431

Estoup, A; Solignac; M; Cornuet; J.M (1994) Precise assessment of the number of patrilines and genetic relatedness in honeybee colonies. Proc R Soc L B 258: 1-7

Free J.B. (1958) The drifting of honeybees. J. Agric. Sci. 51: 294-306

Greeff JM (1996) Effects of thelytokous worker reproduction on kin-selection and conflict in the cape honeybee, *Apis mellifera capensis*. Phil Trans R Soc London B 351(1340): 617-625

Hamilton WD (1964a) The genetical evolution of social behaviour I. J. Theor. Biol. 7:1-16

Hamilton WD (1964b) The genetical evolution of social behaviour II. J. Theor. Biol. 7:17-52

Hepburn R, Reece S, Neumann P, Moritz RFA, Radloff S (1998) Absconding by arrhenotokous and thelytokous honeybees in relation to the role of a colony's queenstate and to the mode of worker reproduction. Submitted to Ins Soc

Jay SC (1969a) The problem of drifting in commercial apiaries. Am Bee J 109: 178-179114

Jay SC (1969b) Drifting of honeybees in commercial apiaries. V. Effect of drifting on honey production. J. Apic. Res. 8: 13-17

Jay SC, Dixon D. (1988) Drifting behaviour and honey production of honeybee colonies maintained on pallets. J. Apic. Res. 27(4):213-218

Keller L, Reeve HK (1994) Genetic variability, queen number and polyandry in social Hymenoptera. Evolution 48: 694-704

Kryger P (1997) The impact of intracolonial genetic relationship on the state of the honeybee family. Ph.D. thesis, Technische Universität Berlin, Germany

Kühl A, Neumann P (1996) Analysis of the drifting of drones at an apiary using single locus DNA-fingerprinting. Apidologie 27 (4): 301-302

Laidlaw HH & Page RE 1984 Polyandry in honey bees (*Apis mellifera* L.): Sperm utilisation and intracolonial genetic relationships. Genetics 108: 985-97

Lecômte J. (1958) Que savons-nous du comportement des faux bourdons?, Ann. Abeille 1, 1, 31-39

Moritz RFA (1988) Der Hobby-Imker. Falken Verlag, Niedernhausen, Germany

Moritz RFA and Neumann P (1996) Genetic analysis of the drifting of drones in *Apis mellifera* using multilocus DNA fingerprinting. Ethology 102, 580-590

Moritz RFA, Southwick EE (1992) Bees as superorganisms. Berlin, Springer Verlag

Moritz RFA, 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

Oldroyd BP, Smolenski AJ, Cornuet JM & Crozier RH (1994) Anarchy in the beehive. Nature 371: 749

Oldroyd BP, Smolenski AJ, Cornuet JM, Wongsiri S, Estoup A, Rinderer TE, Crozier RH (1996) Levels of polyandry and intracolonial genetic relationships in *Apis dorsata* (Hymenoptera: Apidae). Ann Entomol Soc Am 89(2): 276-283

Oldroyd PB, Clifton MJ, Wongsiri S, Rinderer TE, Sylvester HA, Crozier RH (1997) Polyandry in the genus *Apis*, particularly *Apis andreniformis*. Behav Ecol Sociobiol 40: 17-26

Onions G.W. (1912): South African "fertile-worker bees", Agric. J. Union S. Afr. 3, 720-728

Page RE Jr. (1980) The evolution of multiple mating behaviour by honey bee queens (*Apis mellifera* L.). Genetics 96: 263-273

Page RE, Erickson EH (1988) Reproduction by worker honey bees (*Apis mellifera* L.) Behav Ecol Sociobiol 23: 117-126

Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. Heredity 70: 472-480

Poltev VL (1968) [Robbing and drifting of honeybees], Pchelevodsto 88 (7): 42 in Russian. Abstract in Apicultural Abstracts 1973, 24 (2): 78

Ratnieks FLW (1990) The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of removal of diploid males. Behav Ecol Sociobiol 26: 343-348

Ratnieks FLW, Boomsma JJ (1995) Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. Am Nat 145: 969-993

Ratnieks FLW, Visscher P K (1989) Worker policing in the honey bee. Nature 342: 796-797

Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am. Nat. 132: 217-236.

Ratnieks FLW (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. Behav. Ecol. Sociobiol. 32: 191-198.

Ratnieks FLW (1995) Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee. J. Apicult. Res. 34: 31-37.

Rauschmayer F (1928) Das Verfliegen der Bienen und die optische Orientierung am Bienenstand, Arch. Bienenk. 9, 249-322

Roberts WC (1944) Multiple mating of queenbees proved by progeny and flight tests. Glean Bee-Cult 72: 281-283

Ruttner F, Ruttner H (1966) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 3. Flugweite und Flugrichtung der Drohnen. Z Bienenforschung 8: 332-354

Ruttner F, Ruttner H (1972) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 5. Drohnensammelplätze und Paarungsdistanz. Apidol 3: 203-232

Sherman PW, Seeley TD, Reeve HK (1988) Parasites, pathogens and polyandry in social Hymenoptera. Am Nat 131: 602-10

Stuart RJ (1987) Transient nestmate recognition cues contribute to a multicolonial population structure in the ant, *Leptothorax curvispinosus*. Behav Ecol Sociobiol 21, 229-235

Stuart RJ (1988) Development and evolution in the nestmate recognition systems of social insects. In: Evolution of Social Behaviour and Integrative Levels (Ed. By G. Greenberg & E. Tobach), pp. 177-195. Hillsdale, New Jersey: Erlbaum.

Visscher PK (1989) A quantitative study of worker reproduction in queenright honey bee colonies. Behav Ecol Sociobiol 25: 247-254.

Vollbehr J (1975) Zur Orientierung junger Honigbienen bei ihrem ersten Orientierungsflug. Zool. Jb. Physiol. 79:33-69

Waldmann B, Frumhoff PC, Sherman PW (1988) Problems of kin recognition. Trends Ecol Evol 3: 8-13

I

Testing the reliability of the potential honeybee mating area Baltrum using virgin queens and single-locus DNA-fingerprinting

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Summary

24 virgin sister queens were kept for 21 d in mating nuclei on the drone-free island Baltrum to test the reliability of a potential mating area. On each of the neighbouring island mating yards Norderney and Langeoog 12 sister queens were kept with drones. Workers from colonies with island-mated queens (n = 11 Baltrum, n = 7 Langeoog and n = 6 Norderney) were genotyped with four DNA microsatellite loci (n = 996) to estimate the mating frequency of their mother queens. The standard genetic distance and differences in allele frequencies between the populations were determined to estimate putative origins of the drones. The number of matings ranged from 1 to 15 per queen with an average of 6.45 ± 4.2 (effective number 5.39 ± 3.94). 49.30% of the identified drone fathers did not descent from any of the queens on the adjacent islands. They came most likely from mainland colonies at least 5.4 km (3 km across open water) away. It cannot be ruled out that 45.07% of the drones might origin from Langeoog but their allele frequencies were significantly different from the drones which mated with the Langeoog queens. 4 drones showed only alleles found on both mating yards and could not be excluded from any source. High genetic distances (Langeoog/Baltrum D = 0.744, Norderney/Baltrum D = 0.861) and significantly different allele frequencies between the Langeoog and Norderney drone populations and the Baltrum patrilines also indicate that interactions between Baltrum and the neighbouring islands were most unlikely. Our results indicate that successful mating flights are possible on the drone-free island of Baltrum but the low mating frequency suggests that mating conditions were harder than usual. Most likely queens were able to cross at least 5.4 km with more than 3 km of open water at high tide during mating flights.

Key words: *Apis mellifera*, DNA-fingerprinting, mating control, polyandry, population genetics

Introduction

Honeybee queens, *Apis mellifera*, are highly polyandrous which is an uncommon phenomenon among eusocial Hymenoptera (Boomsma & Ratnieks 1996). The estimated degree of polyandry ranges from 5 to 37 according to different authors and methods of investigation (Taber 1954, Triasko 1956, Peer 1956, Woyke 1960, Gary 1963, Adams *et al* 1977, Estoup *et al* 1994 among others). Virgin queens leave their hives for 1 to 5 nuptial flights (Alber *et al* 1955, Roberts 1944), have been shown to mate at a drone congregation area and return to the colony (Ruttner and Ruttner 1966, 1972). The maximum observed mating flight range was 16 km in mainland Canada (Peer 1957). During their flights the queen usually has the possibility to mate with many drones from different colonies and apiaries (Ruttner and Ruttner 1966, 1972).

Achieving control over this complex mating system with its important redistribution of genetic material is certainly one of the key factors of selective honeybee breeding. Artificial insemination techniques have brought the mating of honey bees under complete control. However, this method is labour intensive and technically complex. These are the principal reasons why instrumental inseminations are mainly restricted to scientific oriented breeding programs. The majority of commercial and hobby beekeepers makes instead use of mating yards to achieve control of natural matings. Mainland mating stations are established at isolated places with no or few feral and commercial bee colonies around (Ruttner 1983). With increasing knowledge of honeybee mating behavior the reliability of mainland stations was questioned (Tiesler 1972, Ruttner 1972). In order to ensure controlled matings the following things were recommended: 1. No unselected drones should be allowed within a radius of 10km around the mating yard (Böttcher 1972) 2. increased number of selected drone colonies at the mating yard (Maul 1972) 3. regular requeening of the neighbouring apiaries (Zander & Böttcher 1989). However, total control over matings is not guaranteed at these places.

Since 1923 islands were used in Germany as an alternative tool to achieve controlled matings. After initial problems with cold and windy weather conditions and an underestimation of the number of drone colonies needed, several islands were utilised as routine mating yards. The large areas of open water around these islands are obviously free of bee colonies. Furthermore, Heran & Lindauer (1963) and Heran (1964) showed that open water seems to have a negative impact on the orientation of honeybee workers during their flights. So, islands have been claimed to be ideal places because bees are not expected to cross open water during their mating flights.

In the past bee breeders repeatedly report on uncontrolled matings even on these safe island mating areas. Recent studies of queen honey bee mating behavior on drone-free islands strongly support these observations because they reveal that queens returned from their nuptial flights with a mating sign even during high tide (van Praagh *et al* in press).

Placing virgin queens without drones and displacement experiments of drones have been used for testing mating yards (Zander & Böttcher 1989). However, Ruttner & Ruttner (1963) pointed out that these experiments take place under unusual conditions. A reliability test under normal operating conditions on a mating yard is only possible using markers to separate worker offspring derived from matings with unselected drones. Reliability tests thus used either different races (Böttcher 1947, Ruttner 1959) or mutants like *cordovan* (Ruttner & Ruttner 1965, Drescher 1974). Both methods may suffer from the pitfall of different qualities of the queens and drones involved in the testing procedure. Livenetz (1954) and Drescher (1968) showed differences for drone flight behavior. Koeniger *et al* (1989) found assortative matings resulting from a different vertical distribution of *A. mell. ligustica* and *A. mell. carnica* drones at a natural drone congregation area. For the *cordovan*-test the situation is quite similar. As a result of the highly inbred *cordovan* lines the number of times the *cordovan* queens mated with selected drones might be misinterpreted because of a higher proportion of diploid drones in the resulting *cordovan* offspring. This might occur unless two unrelated *cordovan* strains were used (Peer 1957). Moreover, it has been shown that *cordovan* is not neutral with respect to worker interactions within colonies (Frumhoff 1991) and the flight activity and length of life of wildtype and mutant drones may be different (Witherell 1972). However, Ruttner & Ruttner (1965) and Drescher (1974, unpublished data) found no differences between *cordovan* and the wildtype.

A vast number of mainland mating yards were *cordovan* analysed (Maul 1972), showing that these yards did not guarantee a 100% of mating control. In the case of island mating yards three methods have been used so far to test the reliability of these stations (table I). Especially local characteristics and the distance across open water in between a range of 1 to 10 km towards unselected drone producing colonies seem to play a major role in the reliability of the island mating yards.

These observations call for a genetical control of the reliability of mating yards without interfering with routine bee breeding practice and honeybee behavior. This have never been done before for any of the mating islands currently in use. In this project we decided to test the reliability of island mating stations using virgin queens and single-locus DNA-fingerprinting to asses the number of times honeybee queens mate under drone-free conditions on an island and to determine possible interactions with the neighbouring mating yards.

For that purpose a potential new mating area on the island of Baltrum was examined in the season 1995. Single-locus DNA-fingerprinting enabled us to genotype the worker offspring of the island-mated queens from Baltrum and from the neighbouring island mating yards Langeoog and Norderney. We could estimate the number of times the queens mated if no free flying sexual mature drones are available on Baltrum. Furthermore, we determined the standard genetic distance and the allele frequency differences between the drone populations of Baltrum, Langeoog and Norderney to evaluate the genetic isolation between the islands.

Materials and methods

(a) experimental design

48 virgin sister queens were reared in summer 1995. 24 of them were kept in mating nuclei on Baltrum without drone colonies. On the neighbouring island mating yards Langeoog and Norderney 12 queens each were in the viscidity of 15 (Norderney) or 10 (Langeoog) drone colonies all with sister queens (distances in table II). All virgin queens were allowed to fly during a period of 21 days. The queens of the drone colonies on Norderney were daughters from one mother queen instrumentally inseminated using semen of drones from three sister colonies and the mixed sperm technique. This results in a maximum number of 7 alleles per locus in the worker offspring on this island. On Langeoog a maximum number of 12 alleles was possible per locus. Sealed worker brood samples (n = 50 per queen) of the mated queens were taken (n = 11 for Baltrum, n = 7 for Langeoog and n = 6 for Norderney) and raised isolated in an incubator to exclude mixing. Freshly emerged workers were immediately stored in 96% Ethanol at -15° C.

(b) DNA isolation and microsatellite analysis

DNA was phenol extracted from single workers (n = 40 per colony) following routine protocols (Beye and Reader 1993) with the following changes:

1. Workers were incubated under shaking in insect Ringer solution (127 mM NaCl, 1.5 mM $CaCl_2$, 5 mM KCl, pH 7.4 with NaOH) for one hour at RT before extraction.
2. Single workers thoraces were homogenised in 400 µl of DNA extraction buffer (100 mM NaCl, 100 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.1 % SDS).
3. DNA was resuspended in 30 µl DDH_2O.

We used four DNA-microsatellites which were developed by Estoup *et al* (1993). Multiplex PCR was done using two pairs of loci (A43/B124, A76/A107) and the standard protocols of Estoup *et al* (1993, 1994). Amplification products were electrophorized on 6% polyacrylamide sequencing gels for 5.5h (A76/A107) or 5h (A43/B124) with M13mp18 control DNA sequencing reactions run on the same gel as size standards. Microsatellite alleles were scored as fragment lengths in base pairs.

(c) Data analysis

1. number of effective males

The average intracolonial relatedness $\bar{R}$ was estimated as defined by Estoup *et al* (1994). The number of effective males was then calculated using the equation from Crozier and Pamilo (1996) solved for n_e:

$$n_e = \frac{2}{4\bar{R} - 1} \qquad (1)$$

where n_e = number of effective males and $\bar{R}$ = average intracolonial relatedness.

2. genetic distance

We used the standard genetic distance of Nei (1987):

$$D = -\ln \frac{J_{12}}{\sqrt{J_1 J_2}} \text{with, } j_1 = \sum p_i^2 \text{, } j_2 = \sum q_i^2 \text{, and } j_{12} = \sum p_i q_i \qquad (2)$$

where j_1 is the probability that two randomly chosen genes in population 1 are identical, j_2 is the same for population 2, and j_{12} is the probability that two genes, one drawn randomly from population 1 and the other from population 2, are identical. This set was calculated for each of the four loci. Than the average for all loci was calculated in each of the three cases (J_1, J_2, J_{12}).

d) Genotype analysis and number of matings

The genotypes of the mother queens and the father drones were determined from the genotypes of the sampled workers. The queen was assumed to be homozygous when an allele was present in every worker of the colony. The queen was considered to be heterozygous when every worker carried one of two alleles. The paternal alleles were those not carried by the queen. We used the putative genotype of the mother queen to exclude additional allele combinations. If multiple queen genotypes were possible at a given locus we choose as a rule the allele combination yielding the lowest number of observed matings (n_o).

e) Putative descent of Baltrum drone fathers

The genotypes of all drones who mated with the tested Baltrum queens were compared with the genotypes of the drones which mated with the queens from Langeoog and Norderney. Baltrum drones showing allele combinations that did not correspond with the drone genotypes of one island were excluded from that potential source. Baltrum drone fathers which might origin from the neighbouring island mating yard Langeoog were determined and the differences in allelfrequencies towards the drones which mated with the Langeoog queens were evaluated.

Results

71 paternal genotypes were detected in the 11 sampled Baltrum colonies (table III). We found a range from 1 to 15 patrilines per colony with a mean of 6.45 ± 4.2 matings per queen (table III). The estimates of the effective number of matings on Baltrum ranged from 1 to 14.82 (table III) with a mean of 5.21 ± 4.07. The genotypes of the 13 island-mated sister queens from the neighbouring island mating yards Norderney and Langeoog are given in table IV. The allele frequencies for all tested microsatellite loci are shown in table V. The results of the Bonferroni procedure are found in table VII. For Norderney χ^2-tests were not calculated at the locus A76 because drones could be definitely excluded because of specific alleles. We found for all tested drone populations significant differences for the allele frequencies at least at two loci (table VII). We could exclude 49.30% of the Baltrum drones from any of the drone colonies on the adjacent islands (figure 1). They came either from undetected colonies on Baltrum or more likely from the mainland colonies which were at least 5.4 km away. 45.07% of the drones could have originated from the neighbouring island mating yard Langeoog since they had genotypes in common with the corresponding drones. However, these drones potentially originating from Langeoog, showed significant differences in the allele frequencies of the drones which mated with the queens on Langeoog (Table VII). Four drones (5.63%) showed alleles found on both mating yards and could not be excluded from any source. The standard genetic distances between the tested populations are shown in table VI. High distances were observed between the drones mated with the queens from Langeoog and Norderney and the Baltrum patrilines. A mediate distance was observed between the drones from Norderney and Langeoog whose drone mothers originated from unrelated breeding lines. As expected, low distances were found between the tested sister queens.

Discussion

Our results clearly show that successful mating flights took place on the drone-free island of Baltrum although the next available source of sexual mature drones was at least 5.4 km away. 81.81% of the tested queens mated with males who certainly not originated from the neighbouring island mating yards Langeoog and Norderney. These drones probably derived from colonies on the mainland more than 3 km away, where the mud flats fall dry at low tide. This is the second largest distance ever reported for successful honey bee mating flights across open water after Klatt (7-8 km, 1929). However, Evenius (1931) doubted the drone-free conditions on the peninsula Frisches Haff during that time. In our experiment these drones could also potentially originate from undetected swarms on Baltrum. Since the drone-free conditions were tested using a lure (Gary 1963) and drones could not be attracted on that island (Dustmann *et al* 1996) it seems most likely that the queens fly to the mainland for mating. In light of observations of van Praagh *et al* (in press) it does not seem to play a major role if the mud flats fall dry at low tide or not because queens returned from their nuptial flight with a mating sign even if the tide was high at Baltrum. Virgin queens were able to cross at least 1 km or less across open water as reported by Kramer (1897, cited in Zander & Böttcher 1989), Ruttner & Ruttner (1965) and Klöpping (1993). This shows that such a distance can not prevent honeybee matings. Stored pollen found during the experiments of Ruttner & Ruttner (1965) indicated that even workers were able to cross a 1 km distance across open water during their foraging flights. Given that drones and queens have no lesser flight abilities than workers the important combined mating flight range of queens and drones together (Szabo 1986) found by Evenius (1.2 km, 1929) seem to underestimate the maximum possible mating flight range across open water. Following the observations of Ruttner & Ruttner (1965) one would expect at least 2 km if drones, queens and workers show nearly the same flight ability. On the opposite, successful mating flights of virgin queens of more than 10 km across open water were never reported (Sladen 1920a,b, Minderhout 1923, Evenius 1929, Perkiewicz 1929) in contrast to the mainland (Peer 1957). This shows that distances over open water distinctly decrease the distances needed to reach full genetic isolation of a mating yard. In the range between 1 km and 10 km across open water it is quite uncertain whether mating of virgin queens can occur or not. Klatt (1929a,b) reported of matings 7-8 km across open water. This contrasts to the results of Meinen (1970) for the island of Spiekeroog (7 km distance) and Drescher (1965) for the island of Mellum (6.5-6.9 km distance) who found no returning drones after displacement experiments. However, Ruttner & Ruttner (1963) pointed out that results derived from this kind of experiments should be carefully interpreted. The situation of displaced drones and well feed drones which are on their mating flights could be very different. Moreover, displaced drones can return only by chance since they have no knowledge of their hive location. On the other hand virgin queens were not mated on the island of Mellum (Drescher 1965). However, the queens had the possibility to mate only during a 6 d period and even on the mainland queens need up to 15 d to mate with drones from colonies 6 km away (Peer & Ferrar 1956). We found after a period of 21 d successful matings over a 3 km distance across open water. Oortman Gerling (1993) reported that virgin Buckfast queens were not able to cross 4 km of open water during a 44 d period although the tested island of Marken was connected with the mainland via a dam which might have enhanced honeybee orientation (Heran 1964). Since the queens in our study were able to cross a 3 km water gap without a dam, we cannot exclude that racial characteristics for flight behavior as shown for drones (Livenetz 1954, Drescher 1968, Koeniger *et al* 1989) are also responsible for Buckfast queens having more difficulties to cross open water. Furthermore, various environmental factors like weather conditions during the experiments (Meinen 1970, Englert 1972), preferred wind directions on the area or other local characteristics like vegetation and size of the tested island (Ruttner & Ruttner 1965) or even individual characteristics of queens (Englert 1972) might be responsible for the mating success.

It is important to know if an established mating yard Baltrum might interfere with the neighbouring established yards Langeoog and Norderney. In order to quantify a possible gene flow between these islands we excluded drones from the potential sources Norderney and Langeoog. The allele combinations of the drones who mated with the Baltrum queens enabled us to exclude 49.3% of them from any of the used queens. Given that the queens fly across open water 9 of 11 mated queens certainly interacted with mainland drone sources. An interaction with Norderney seems to be unlikely in most cases because we could exclude the majority of the Baltrum patrilines from that origin (94.37%). Furthermore, the potential "Norderney" drones which mated with the Baltrum queens showed alleles (either 127 bp for locus A43 or 291 bp for locus A76) which were very rare in the patrilines on Norderney. Only two queens showed possible Norderney patrilines in their progeny. In these cases interactions with at least two different drone sources (Norderney and mainland or Norderney, Langeoog and mainland) must have been occurred. Given that queens were searching for drones this seems to be most unlikely. It cannot be ruled out that 45.07% of the drones might origin from Langeoog. Two queens showed only potential Langeoog progeny in their worker offspring. However, we found significant different allele frequencies between these potential "Langeoog" drones and the drones which mated with the Langeoog queens. We therefore reject the hypothesis that the drones originated from the same gene pool. Following the argumentation given for Norderney we consider it unlikely that the queens had visited two possible drone sources during their mating flights. The standard genetic distances also indicate that matings with drones from the neighbouring island mating yards were most unlikely. The distances between the drones which mated with the Langeoog and Norderney queens and the Baltrum patrilines were distinctly higher than the distances between the unrelated drone populations of Norderney and Langeoog.

The neighbouring island mating yards Langeoog and Norderney were provided with drone colonies. No additional alleles were determined for the locus A76 on Norderney and we found 10 out of maximal 12 possible alleles for the same locus on Langeoog. These results are in line with the expectation that drones do not drift among islands. In favour of an active role of the queens in this special case of drone-free islands are observations of Dustmann *et al* (1996) that drones attracted by a lure on the mainland near Baltrum did not follow it across the mainland border and of Ruttner & Ruttner (1963) who never attracted drones on a lake.

The mating frequency of the Baltrum queens had a surprisingly high variance. At the average the mated queens showed a distinctly smaller number of observed and effective matings as natural mated queens in other populations (Adams *et al* 1977, Estoup *et al* 1994, Neumann *et al* in review). The number of times the queens mated was certainly depend upon the low drone density (Ruttner 1959, Tiesler 1972, Szabo 1986) and the distances to the next available drone colonies. Our data clearly shows in addition to unusual long lasting flights and a delayed egg laying (van Praagh *et al* in press) that mating conditions for virgin queens on drone-free areas like Baltrum are much more difficult than it is the normal case on an island mating area. These results are in line with Szabo (1986) who found for mated sister queens on the mainland correlations between the distance towards drone colonies, the number of spermatozoa and the time of starting oviposition.

As a result of this we can obviously not give a judgement of the reliability of Baltrum as an established mating yard. A reliability testing is only possible under normal bee keeping conditions with a sufficient number of drone colonies (Ruttner 1959, Drescher 1974). It seems unlikely that queens would mismate if an adequate number of drones is available on Baltrum. However, our results clearly show that queens would have the possibility of successful interactions with mainland drone sources. Nevertheless, controlled matings cannot be guaranteed on the potential mating area Baltrum at the current state of evidence.

We showed that a reliability test using single-locus DNA-fingerprinting has important advantages: 1. In contrast to all other methods used so far, single-locus DNA-fingerprinting can be incorporated in the routine procedure at a mating yard, thus drones and queens which were normally used on these yards can be tested. 2. Small worker samples are needed from the tested colonies which can easily be taken by the beekeeper. 3. The origin of the unselected drones which mated with the tested queens can be found. 4. No efforts of breeding and establishing *cordovan* lines. 5. The number of times the queens mated with unselected drones can be exactly determined. As in the case of the mainland mating yards island mating stations laying within the maximum reported mating flight range of 7-8 km across open water (Klatt 1929a,b) should be examined in a similar manner. Even at isolated places like islands which are normally free of bees it can not be *a priori* guaranteed that natural mating is under complete control.

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Detailed summary

24 drohnenfreie Völker mit unbegatteten Geschwisterköniginnen wurden 1995 für 21 Tage auf der Drohnen freien Insel Baltrum plaziert, um die Zuverlässigkeit einer potentiellen Belegstelle zu testen. Dazu wurde erstmalig ergänzend das Verfahren des single-locus DNA-fingerprinting angewandt (Tabelle I). Auf jeder der benachbarten Inselbelegstellen Langeoog und Norderney standen je 10 weitere Geschwisterköniginnen mit geschlechtsreifen Drohnen (Distanzen siehe Tabelle II). Aus jedem Volk mit einer begatteten Königin (n= 11 Baltrum; n= 7 Langeoog und n = 6 Norderney) wurden Arbeiterinnenbrutwaben entnommen. Isolierte, frisch geschlüpfte Arbeiterinnen wurden mit single-locus DNA-fingerprinting unter Verwendung vier verschiedener Loci genotypisiert (n = 996), um die beobachtete und effektive Paarungshäufigkeit der Baltrumköniginnen (Tabelle III) und die vermutliche Herkunft der Drohnen zu ermitteln. Dazu wurden die Genotypen der getesteten Geschwisterköniginnen (Tabelle IV) und der auf Baltrum, Langeoog und Norderney angepaarten Drohnen (Tabellen III und V) ermittelt. Zwischen den getesteten Populationen wurden die genetischen Distanzen nach Nei berechnet (Tabelle VI) und mögliche Unterschiede in den Allelfrequenzen mit Hilfe der verbesserten Bonferroni Methode untersucht (Tabelle VII). 71 Paarungen wurde für Baltrum nachgewiesen (Tabelle III). Die Anzahl an beobachteten Paarungen pro Königin lag zwischen 1 und 15 mit einem Mittelwert von 6,33 ± 4,29. Die mittlere Anzahl effektiver Paarungen von 5,85 ± 4,05 (Tabelle III) war deutlich geringer als die Ergebnisse früherer Studien an natürlich gepaarten Königinnen (z.B. n_e = 17.25; Adams *et al* 1977). Die ermittelten Drohnen konnten aufgrund ihrer Allelkombinationen von Herkünften ausgeschlossen werden (Abbildung 1). 49,30% der identifizierten Drohnen stammten nicht von den eingesetzten Königinnen. Sie kamen entweder von unentdeckten Schwärmen auf Baltrum oder von Völkern auf dem Festland, die mindestens 5,4 km (davon 3 km über offenes Wasser) entfernt lagen. Es kann nicht ausgeschlossen werden, daß 45,07% der

Drohnen von Langeoog stammen. Diese Drohnen zeigten jedoch signifikant unterschiedliche Allelfrequenzen zu den Drohnen, mit denen sich die Königinnen auf Langeoog gepaart haben (Tabelle VII). Vier Drohnen wiesen auf beiden Belegstellen vorkommende Allele auf und konnten somit von keiner Quelle ausgeschlossen werden. Gegen eine Herkunft von Norderney spricht jedoch, daβ diese Drohnen Allele aufwiesen, die auf Norderney sehr selten waren, entweder 127bp für den Locus A43 oder 291bp für den Locus A76 (Tabelle III). Eine Herkunft der Drohnen von den eingesetzten Königinnen auf Baltrum konnte ausgeschlossen werden, da eine Entwicklung geschlechtsreifer Drohnen zwischen der Aufstellung der Begattungsvölkchen und dem Rücktransport der Königinnen nicht erfolgen konnte. Hohe genetische Distanzen (Tabelle VI) und signifikant unterschiedliche Allelfrequenzen (Tabelle VII) zeigten die angepaarten Drohnen von Langeoog und Norderney und die Patrilinien von Baltrum. Dies deutet ebenfalls daraufhin, daß Paarungen zwischen Drohnen von Langeoog oder Norderney und den Königinnen von Baltrum unwahrscheinlich sind. Für die benachbarten Inselbelegstellen Norderney und Langeoog konnten im Rahmen unserer Stichprobe keine Paarungen mit unselektierten Drohnen nachgewiesen werden. Da jedoch von den dort aufgestellten Drohnenmüttern keine Genotypendaten vorlagen, konnte dies nicht mit Sicherheit ausgeschlossen werden. Die im Gegensatz zu natürlich gepaarten Königinnen geringere Paarungshäufigkeit zeigt im Zusammenhang mit verspäteter Eilage und ungewöhnlich langen Ausflugzeiten (van Praagh *et al*, in press) deutlich, daß die Paarungsbedingungen auf der drohnenfreien Insel Baltrum als erschwert zu werten sind. Ob sich Königinnen unter regulären Bedingungen auf einer etablierten Belegstelle Baltrum, d.h. mit einer ausreichenden Anzahl von selektierten Drohnenvölkern, auch mit Drohnen vom Festland paaren, bleibt offen, da das Aufstellen unbegatteter Königinnen ohne Drohnenvölker nicht für die Einschätzung der Zuverlässigkeit einer Belegstelle unter Routinebedingungen geeignet ist. Unsere Ergebnisse demonstrieren jedoch eindeutig, daß auf Baltrum kontrollierte Paarungen nicht *a priori* garantiert werden können. Entweder Drohnen oder Königinnen oder beide Geschlechter sind in der Lage, bei ihren Paarungsflügen größere Strecken offenen Wassers zu überqueren (Tabelle II). Das von uns angewandte Verfahren des single-locus DNA-fingerprinting ermöglicht eine Überprüfung der Sicherheit von Belegstellen im Routinebetrieb unter Verwendung des dort üblichen Zuchtmaterials. Eine derartige Überprüfung anderer, bereits etablierter Belegstellen wäre wünschenswert.

References

Adams J, Rothmann ED, Kerr WE, Paulino ZL (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

Alber M, Jordan R, Ruttner F, Ruttner H (1955) Von der Paarung der Honigbiene, Z Bienenforsch 3, 1-28

Beye M, Raeder U (1993) Rapid DNA preparation from bees and % GC fractionation, Bio Techniques, 14, 3, 372-374

Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. Phil Trans R Soc B 331, 947-975

Böttcher FK (1947) Was taugt die Belegstelle? Der Imkerfreund 2, 115-116

Böttcher FK (1972) Richtige Führung einer Landbelegstelle. In: Paarungskontrolle und Selektion bei der Honigbiene (Ruttner F, Ruttner H, Harnaj V), Apimondia, Bukarest

Crozier RH, Pamilo P (1996) Evolution of social insect colonies, Oxford University Press, Oxford

Drescher W (1965) Untersuchungen zur Zuflugsicherheit der Inselbelegstelle Mellum. Z Bienenforsch 8 (3), 49-53

Drescher W (1968) Die Flugaktivität von Drohnen der Rasse *Apis mellifera* carnica L und *A. mell. ligustica* L in Abhängigkeit von Lebensalter und Witterung, Z Bienenforsch 9 (9), 390-409

Drescher W (1974) Betrachtungen zum Thema Belegstellen. ADIZ (8), 57-62

Dustmann JH, van Praagh JP, Faltus E, Maarzahl H (1996) Die Insel Baltrum als Belegstelle?, DBJ 4(6), 266

Englert E (1972) Versuche und Beobachtungen auf der Inselbelegstelle Neuwerk. In: Paarungskontrolle und Selektion bei der Honigbiene (Ruttner F, Ruttner H, Harnaj V), Apimondia, Bukarest

Estoup A, Solignac M, Harry M, Cornuet JM (1993) Characterisation of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. Nucleic Acids Res 21 1427-1431

Estoup A, Solignac M, Cornuet JM (1994) Precise assessment of the number of patrilines and genetic relatedness in honeybee colonies, Proc R Soc Lond [Biol] 258 1-7

Evenius J (1929) Königinnenzuchtversuche auf der Greifswalder Oie. Leipz Bztg 44 (12), 244

Evenius J (1930) Zur Frage der Belegstellen. Westf BZ 45, 293-294

Evenius J (1931) Die neuzeitliche Bienenzucht und die Verwendung von Inseln als Belegstellen. Bienenwirtsch Zentr (4), 92-95

Frumhoff PC (1991) The effect of the *cordovan* marker on apparent kin discrimination among nestmate honeybees, Anim Behav 42, 854-856

Gary NE (1963) Observations of mating behaviour in the honeybee, J Apic Res 2, 3-13

Heran H (1964) Wie überwacht die Biene ihren Flug? Umschau (64) 10, 299-303

Heran H, Lindauer M (1963) Windkompensation und Seitenwindkorrektur bei Flug über Wasser. Z vgl Physiol 47 (39), 39-55

Hochberg Y (1988) A sharper Bonferroni procedure for multiple tests of significance. Biometr 75, 800-802

Klatt G (1929a) Züchtungsmöglichkeiten an der Wasserkante. AfB X 8, 318-321

Klatt G (1929b) Unsere ostpreußischen Belegstellen. Die deutsche Bienenzucht in Theorie und Praxis 37 (3), 74-77

Klöpping B (1993) Hompelvoet is getest. Buckfast Bulletin Nr. 16, 15

Koeniger G, Koeniger N, Pechhacker H, Ruttner F, Berg S (1989) Assortative mating in a mixed population of European honeybees, *Apis mellifera ligustica* and *Apis mellifera carnica*. Ins Soc (36), 129-138

Livenetz IP (1954) [the flight range of drones] Pchelovodsto 8, 36-38 (abstract in Bee World 36), 59

Maul V (1972) Belegstellen-Prüfungen im *cordovan*-Test. In: Paarungskontrolle und Selektion bei der Honigbiene (Ruttner F, Ruttner H, Harnaj V), Apimondia, Bukarest

Meinen G (1970) Wie sicher ist die Inselbelegstelle Spiekeoog vor fremdrassigem Drohnenzuflug? NWDIZ (22), 128-130

Minderhoud M (1923) Verslag der proeven over koniginnenentelt op Urk. Maandschrift voor Bijeenteelt 26 (3), 40

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Oortman Gerling E (1993) Betrouwbaarheids-proef op Marken 1992, Buckfast Bulletin 15, 31-32

Pauls (1929) Wir von der Wasserkante und unsere Belegstellen. Leipzig Bztg 44, 174

Perkiewiecz E (1929) Beitrag der Imkerschule zur Belegstellenfrage. Leipzig Bztg 44, 174

Peer DE (1956) Multiple mating of queen honey bees. J Econ Entomol (49), 741-743

Peer DE (1957) Further studies on the mating range of the honeybee, *Apis mellifera* L.. Can Entomol 89, 108-110

Peer D, Ferrar CL (1956) The mating range of the honey bee. J Econ Entomol 49 (2), 254-256

Ruttner F (1959) Drohnenflugweite und Drohnendichte. Deutsche Bienenwirtsch 10 (1), 42-47

Ruttner F (1983) Zuchttechnik und Zuchtauslese bei der Biene: Anleitungen zur Aufzucht von Königinnen und zur Kör- und Belegstellenpraxis. Ehrenwirth Verlag, München

Ruttner F, Ruttner H (1965) Wie weit fliegen Drohnen und Königinnen?, Bienenvater 86 (1), 15-21

Ruttner F, Ruttner H (1966) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 3. Flugweite und Flugrichtung der Drohnen, Z Bienenforsch 8, 332-354

Ruttner F, Ruttner H (1972) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 5. Drohnensammelplätze und Paarungsdistanz, Apidol 3, 203-232

Sladen FWL (1920a) Why young queens may fail. Gleanings Bee Cult 48 (2), 50-52

Sladen FWL (1920b) Queen mating experiments. Ebenda 48 (12), 717-718

Simes RJ (1986) An improved Bonferroni procedure for multiple tests of significance, Biometr 73, 751-754

Szabo T (1986) Mating distances of the honeybee in north-western Alberta Canada. J Apic Res 25, 4, 227-233

Taber S (1954) The frequency of multiple mating of queen honey bees, J Econ Entomol 47, 995-998

Tiesler FK (1972) Die Inselbelegstellen aus dem Norden der BRD. In: Paarungskontrolle und Selektion bei der Honigbiene (Ruttner F, Ruttner H, Harnaj V), Apimondia, Bukarest

Triasko VV (1956) Polyandry in honey bees, XVI. Int Beekeeping Congr Prelim Sci Meet, abstract in Apic Abstr (233), 56

Verbeek B (1976) Untersuchungen der Ausflugsaktivität von jungen Bienenköniginnen unter Festlands- und Inselbedingungen mittels lichtelektronischer Überwachung. Apidol 7 (2), 151-168

Witherell PC (1972) Flight activity and natural mortality of normal and mutant drone honeybees, J Apic Res 11, 2, 65-75

Woyke J (1960) [natural and artificial insemination of queen honeybees], Pszczel Zesz Nank 4, abstract in Bee World 43, 21-25

Zander E, Böttcher FK (1989) Haltung und Zucht bei der Biene, Eugen Ulmer Verlag, Stuttgart

Figure 1: Excluded descent of the detected father drones on Baltrum

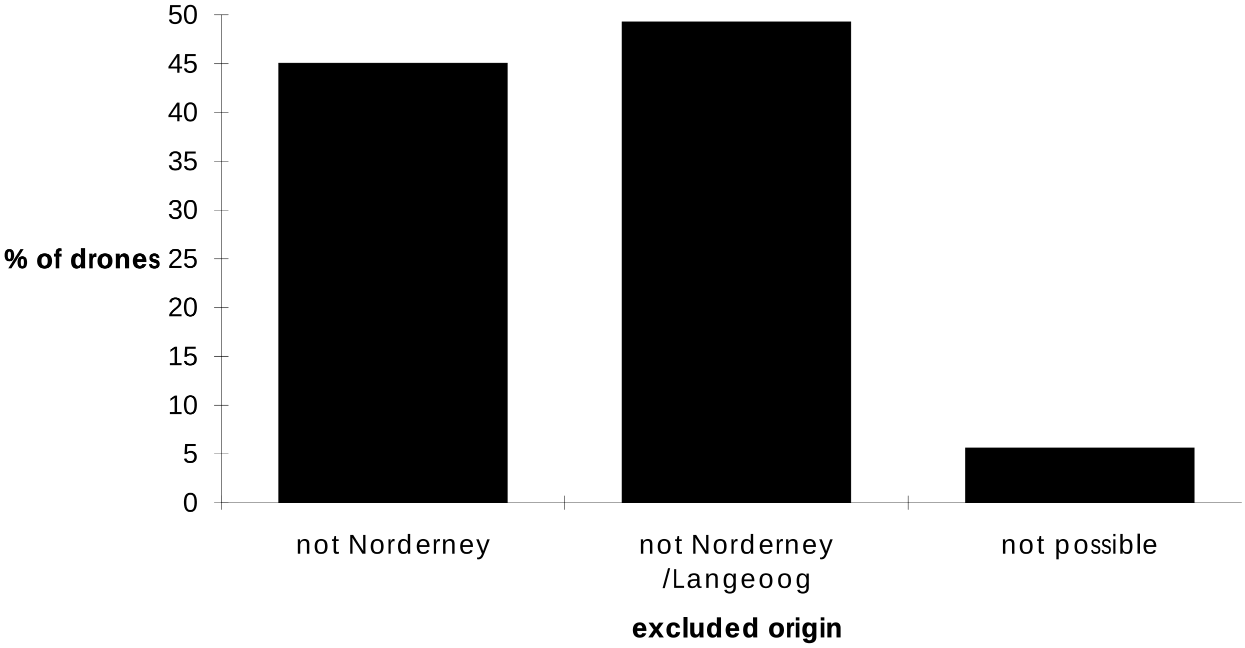

Table I: Methods used for testing the reliability of island or peninsula mating yards (* = connected with the mainland via a dam, ** = actual 2 km but 1 km with dense reed vegetation, d = drones, q =queens, w = workers).

method	yard	distance over open water	uncontrolled mated queens	author(s)
virgin queens	Ufenau	1-4 km	+	Kramer (1897)
	Duk	11.6 km	-	Sladen (1920a,b)
	Urk	18 km	-	Minderhout (1923)
	Greifswalder Oie	10.5 km	-	Evenius (1929)
	Frische Nehrung	7-8 km	+	Klatt (1929a,b)
	Hela	14 km	-	Pauls (1929)
	Trischen	10 km	-	Perkiewicz (1929)
	Mellum	6.5-6.9 km	-	Drescher (1965)
	Marken	4 km*	-	Oortman Gerlings (1993)
	Hompelvoet	600 m	+	Klöpping (1993)
cordovan-test	Neusiedler See	1 km**	+	Ruttner & Ruttner (1965)
displacement		no return	analysed	
	Greifswalder Oie	800 m (d) 400 m (q)	q, d, w	Evenius (1930)
	Mellum	6.5-6.9 km	d	Drescher (1965)
	Spiekeroog	7 km	d	Meinen (1970)

Table II: Distances of the tested Baltrum colonies to the drone colonies on the neighbouring island mating areas and on the mainland (* = shortest distance to any possible source of males)

	Baltrum	
	total distance	distance over open water
Mainland	5.4 km*	3 km
Langeoog	7.8 km	1.4 km
Norderney	13.2 km	750 m

Table III: Putative genotypes (four microsatellite loci, length in base pairs) of the mated Baltrum queens (B1-B11) and the drone fathers and the effective number of queen matings (q = Both alleles were present in the mother queen. It was not possible to determine the allele of the actual father drone; N = number of workers per patriline, n_e = number of effective matings).

Colony	B1					B2					B3				
Sample size					N					N					N
Locus	A107	A76	A43	B124		A107	A76	A43	B124		A107	A76	A43	B124	
Queen's alleles	141	287	140	214		165	283	140	214		141	265	127	216	
	170	291	140	216		176	283	140	216		176	291	140	222	
Patrilinie															
1	163	289	127	214	40	171	271	127	216	24	160	313	q	214	3
2						171	287	127	216	15	160	313	q	228	4
3											162	313	q	214	6
4											162	313	q	228	7
5											164	271	127	214	28
6											170	271	140	214	24
n_e	1			Σ	40	n_e	1.95		Σ	39	n_e	3.66		Σ	72

Colony	B4					B5					B6				
Sample size					N					N					N
Locus	A107	A76	A43	B124		A107	A76	A43	B124		A107	A76	A43	B124	
Queen's alleles	165	265	140	216		165	283	127	216		165	265	140	216	
	170	291	142	222		176	283	140	216		176	283	140	230	
Patrilinie															
1	161	271	140	214	2	165	209	127	216	17	169	265	127	214	4
2	161	271	140	216	2	171	287	q	216	23	169	265	127	230	7
3	170	287	140	214	5						169	265	127	228	2
4	170	287	140	216	4						169	265	127	220	2
5	170	291	140	214	9						169	265	127	232	3
6	170	291	140	q	2						169	271	127	232	1
7	171	229	140	q	4						171	265	127	214	1
8	171	229	140	214	13						171	271	127	214	4
9											171	271	127	216	2
10											171	271	127	230	3
11											171	271	127	222	2
12											171	287	127	214	2
13											171	287	127	228	1
14											171	287	127	230	4
15											171	287	127	232	1
n_e	5.9			Σ	41	n_e	2.00			40	n_e	14.82		Σ	39

Colony	B7					B8					B9				
Sample size					N					N					N
Locus	A107	A76	A43	B124		A107	A76	A43	B124		A107	A76	A43	B124	
Queen's alleles	167	265	140	214		163	265	127	214		165	265	140	214	
	176	283	142	228		170	283	142	216		176	283	142	228	
Patrilinie															
1	171	249	140	216	6	141	249	140	222	5	165	209	127	220	3
2	171	249	140	222	7	141	249	q	224	1	165	209	127	222	3
3	171	271	140	216	10	141	271	140	222	5	165	209	127	228	2
4	171	271	140	222	6	141	271	140	224	2	165	209	127	230	1

5	171	q	140	216	2	176	249	140	222	4	165	271	127	228	2
6	173	249	140	216	5	176	249	140	224	6	165	271	127	230	3
7	q	271	140	222	4	176	271	140	222	1	170	209	127	220	4
8						176	271	140	224	7	170	271	127	220	8
9						q	249	140	222	1	170	271	127	222	5
10						q	249	140	224	3	170	271	127	230	1
n_e	6.9			Σ	40	n_e	9.02		Σ	35	n_e	9.02		Σ	32
Colony	B10					B11									
Sample size					N					N					
Locus	A107	A76	A43	B124		A107	A76	A43	B124						
Queen's alleles	165	283	127	216		141	265	127	214						
	170	291	140	222		176	291	140	216						
Patrilinie															
1	159	271	q	214	1	159	313	q	222	8					
2	170	313	q	214	2	159	313	q	228	9					
3	170	313	q	224	1	166	313	q	222	13					
4	170	313	q	228	11	166	313	q	228	8					
5	171	291	q	214	9										
6	171	291	q	228	10										
n_e	4.09			Σ	34	n_e	4.14		Σ	38					

Table IV: Putative genotypes (four microsatellite loci, length in base pairs) of the mated queens on Langeoog (L1-L7) and Norderney (N1-N6), (/ = both alleles were possible) and sample size per colony (n).

Colony	Locus					Colony	Locus				
	A107	A76	A43	B124	n		A107	A76	A43	B124	n
L1	265	165	127	214	12	N1	265	165	140	216	35
	283	176	140	216			291	170	140	222	
L2	287	165	127	216	33	N2	265	141	140	216	41
	291	170	140	222			291	170	142	222	
L3	283	141	140	216	48	N3	265	141	140	214	43
	291	170	142	220			291	170	142	216	
L4	265	165	140	216	57	N4	265	141	140	216	25
	283	176	140	222			283	176	142	228	
L5	265	141	140	214	51	N5	283	141	140	216	8
	283	176	142	216			291	176	142	214/222	
L6	283	141	140	214	49	N6	265	141	140	214	52
	291	170	140	228			291	176	142	216	
L7	265	165	140	214	52						
	283	176	140	216							

Table V: Allelefrequencies for Baltrum (B), Norderney (N), Langeoog (L), four microsatellite loci, allele size in base pairs. Only sexual reproductives are considered.

A 76																	
Allele	Σ	209	229	249	265	271	281	283	287	289	291	299	307	313	351	353	357
N queens	12	0	0	0	0.417	0	0	0.167	0	0	0.417	0	0	0	0	0	0
N drones	70	0	0.186	0	0.271	0	0.014	0	0	0	0.043	0	0	0	0.014	0.243	0.229
L queens	14	0	0	0	0.286	0	0	0.429	0.071	0	0.214	0	0	0	0	0	0
L drones	89	0	0	0	0.236	0.157	0	0.028	0.006	0.079	0.034	0.337	0.011	0.1124	0	0	0
B queens	22	0	0	0	0.318	0	0	0.409	0.045	0	0.227	0	0	0	0	0	0
B drones	71	0.085	0.028	0.127	0.092	0.324	0	0.007	0.113	0.014	0.056	0	0	0.155	0	0	0

A 107															
Allele	Σ	141	159	160	161	162	163	164	165	166	167	170	171	173	176
N queens	12	0.417	0	0	0	0	0	0	0.083	0	0	0.333	0	0	0.167
N drones	70	0.057	0	0	0	0	0.114	0	0.193	0	0	0.343	0	0	0.293
L queens	14	0.214	0	0	0.071	0	0	0	0.214	0	0	0.2143	0	0	0.286
L drones	89	0.039	0.112	0	0.202	0	0.18	0	0.101	0	0	0.309	0.011	0	0.045
B queens	22	0.136	0	0	0	0	0.045	0	0.273	0	0.045	0.181	0	0	0.318
B drones	71	0.056	0.042	0.028	0.028	0.028	0.028	0.014	0.099	0.028	0.007	0.268	0.296	0.014	0.063

B 124											**A 43**								
Allele	Σ	214	216	218	220	222	224	228	230	232	Σ	126	127	128	132	139	140	141	142
N queens	12	0.25	0.5	0	0	0.167	0	0.083	0	0	12	0	0	0	0	0	0.75	0	0.25
N drones	70	0.372	0.136	0	0	0.236	0	0.257	0	0	69	0	0.029	0.261	0	0.058	0.420	0.029	0.203
L queens	14	0.286	0.429	0	0.071	0.143	0	0.071	0	0	14	0	0.071	0.071	0	0	0.714	0	0.143
L drones	80	0.225	0.394	0.163	0.169	0.038	0	0.013	0	0	88	0.023	0.097	0.063	0.023	0	0.608	0	0.188
B queens	22	0.272	0.454	0	0	0.136	0	0.091	0.045	0	22	0	0.227	0	0	0	0.591	0	0.182
B drones	71	0.169	0.176	0	0.056	0.246	0.085	0.141	0.085	0.042	71	0	0.803	0	0	0	0.190	0	0.007

Table VI: Standard genetic distances between the tested populations

	Baltrum queens	Langeoog queens	Norderney queens		Baltrum drones	Langeoog drones	Norderney drones
Baltrum queens	0	0.029	0.125	Baltrum drones	0	0.744	0.861
Langeoog queens	0.029	0	0.084	Langeoog drones	0.744	0	0.414

Table VII: Improved Bonferroni procedure for dependent test statistics (*p(i)* = ordered *p*-values for χ2-tests for allelefrequencies between the drone populations mated with queens from Baltrum, Norderney and Langeoog and for the potential Langeoog drones which mated with Baltrum queens, *α(i)* = significance level for the subhypothesis H_i, four microsatellite loci, allele size in base pairs). Significant differences ($p_{(i)} < \frac{\alpha}{n-i+1}$) for a given locus or allele where indicated through *.

Langeoog/Baltrum

A 76*

Allele	283	307	291	289	265	313	229	209*	271*	287*	249*	299*
p(i)	0.434	0.415	0.183	0.126	0.107	0.066	0.040	0.00038	0.0002	< 0.001	< 0.001	< 0.001
rank	12	11	10	9	8	7	6	5	4	3	2	1
α(i)	0.05	0.025	0.017	0.013	0.01	0.008	0.007	0.006	0.00556	0.005	0.00455	0.00417

A 107*

Allele	165	170	141	176	167	164	173	159	160	162	166	163	161*	171*
p(i)	0.96	0.63	0.621	0.615	0.429	0.263	0.263	0.122	0.113	0.113	0.113	0.005	0.002	< 0.001
rank	14	13	12	11	10	9	8	7	6	5	4	3	2	1
α(i)	0.05	0.025	0.01667	0.0125	0.01	0.00833	0.00714	0.00625	0.00556	0.005	0.00455	0.00417	0.00385	0.00357

B 124*

Allele	214	232	220	216	224	230	222*	228*	218*
p(i)	0.996	0.066	0.043	0.013	0.009	0.009	0.005	0.004	< 0.001
rank	9	8	7	6	5	4	3	2	1
α(i)	0.05	0.025	0.01667	0.0125	0.01	0.00833	0.00714	0.00625	0.00556

A 43*

Allele	126	132	128	142*	140*	127*
p(i)	0.20398	0.20398	0.03516	0.00054	5.5E-05	5.39E-12
rank	6	5	4	3	2	1
α(i)	0.05	0.025	0.01667	0.0125	0.01	0.0083

Norderney/Baltrum

A107*

Allele	141	167	170	164	173	160	161	162	166	165	159	163	176*	171*
p(i)	0.984	0.483	0.419	0.321	0.321	0.160	0.160	0.160	0.160	0.142	0.085	0.055	0.001	< 0.001
rank	14	13	12	11	10	9	8	7	6	5	4	3	2	1
α(i)	0.05	0.025	0.01667	0.0125	0.01	0.00833	0.00714	0.00625	0.00556	0.005	0.00455	0.00417	0.00385	0.00357

B 124

Allele	222	216	228	214	232	220	224	230
p(i)	0.557	0.544	0.121	0.112	0.085	0.047	0.015	0.015
rank	8	7	6	5	4	3	2	1
α(i)	0.05	0.025	0.01667	0.0125	0.01	0.00833	0.00714	0.00625

A 43*

Allele	141	139	140*	142*	128*	127*
p(i)	0.151	0.042	0.013	< 0.001	< 0.001	< 0.001
rank	6	5	4	3	2	1
α(i)	0.05	0.025	0.01667	0.0125	0.01	0.00833

Langeoog/potential Langeoog

A 76*											
Allele	283	307	265	291	289	271	287*	299*	287*	249*	299*
p(i)	0.624	0.522	0.185	0.119	0.091	0.011	0.002	< 0.001	< 0.001	< 0.001	< 0.001
rank	11	10	9	8	7	6	5	4	3	2	1
$\alpha(i)$	0.05	0.025	0.01667	0.0125	0.01	0.00833	0.00714	0.00625	0.00556	0.005	0.00455

A 107*									
Allele	176	141	159	170	167	165	161	163	171*
p(i)	0.991	0.818	0.747	0.64	0.25	0.225	0.086	0.015	< 0.001
rank	9	8	7	6	5	4	3	2	1
$\alpha(i)$	0.05	0.025	0.01667	0.0125	0.01	0.00833	0.00714	0.00625	0.00556

B 124							A 43*						
Allele	214	220	216	218	222	228	Allele	140	126	132	128	142	127*
p(i)	0.301	0.16	0.101	0.021	0.003	0.001	*p(i)*	0.489	0.386	0.386	0.151	0.013	< 0.001
rank	6	5	4	3	2	1	rank	6	5	4	3	2	1
$\alpha(i)$	0.05	0.025	0.01667	0.0125	0.01	0.00833	$\alpha(i)$	0.05	0.025	0.01667	0.0125	0.01	0.00833

II

Queen mating-frequency on different types of honeybee mating yards

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Summary

Worker samples were taken from 27 queenright honeybee colonies. The queens of these colonies were mated on two island mating yards (N = 7 Langeoog; N = 6 Norderney), on two low land yards (N = 6 Gramschatzer-Wald; N = 4 Königswald), on a highland mating yard (N = 3 Rachel-Diensthütte) and on an isolated high mountain station (N = 3 Hochgrat). Individual workers (N = 1055) were genotyped using four DNA microsatellite loci and the observed (n_o) and effective (n_e) numbers of matings were estimated from the worker offspring. The observed number of matings per queen ranged from 6 to 24 (n_e = 4.61 to 31.07). Significant differences were found between island and mainland mating yards with an average of $n_o = 13 \pm 1.14$ ($n_e = 11.82 \pm 1.19$) for the two islands and a mean of $n_o = 18.06 \pm 1.08$ ($n_e = 20.46 \pm 1.65$) for the mainland yards. No differences were observed between the tested island and among the tested mainland mating yards. The number of drone colonies at the tested mating yards showed no significant effect on the queen mating frequency. This indicates that distinct local climate conditions on the island mating yards are most likely to have a negative impact on queen mating frequency in our sample.

Key words: *Apis mellifera*, climate, DNA-microsatellite, environment, island mating yard, mainland mating yard, mating condition, multiple mating, population genetics, weather

Introduction

The control over matings of virgin queens is one of the inherent complexities of honeybee breeding that were responsible for a disappointing progress in the past (Laidlaw & Page 1986). The development and improvement of artificial insemination techniques gave complete control over the mating of honeybee queens and "opened a wide door to both breeding and genetics" (Cale and Rothenbuhler 1975). However, this wide door has been mainly used by honeybee genetics in scientifically oriented breeding programs. The vast majority of commercial honeybee breeders tries to achieve mating control through mating yards. Mating yards are ideally established at isolated places like deep forests, mountains or islands with no or few feral honeybee colonies around (Ruttner 1983). Commercial beekeeping of unselected strains should not be allowed within a radius of 10 km around these mating yards (Böttcher 1972).

After initial setbacks due to a limited number of drone colonies (Tiesler 1972) island mating yards were believed to yield the best natural mating control. Nevertheless the mating success is low which is often attributed to poor weather conditions for honeybee mating (Alber *et al* 1955, Meinen 1970, Englert 1972, Tiesler 1972, Verbeek 1976). Moreover, local characteristics of the mating yards such as the position and number of the mating nuclei and the drone colonies, the yard location or the position of the hive entrances towards the sun have also been claimed to effect mating success (Englert 1974, Zander & Böttcher 1989).

So far mating success has only been analyzed through the proportion of successfully mated and egglaying queens. However this seems to be a very rough measure for mating success in light of the high degree of polyandry of honeybee queens. The recent advances in DNA technology in honeybee genetics (Moritz *et al* 1991, Fondrk *et al* 1993, Estoup *et al* 1993) allow to analyze mating efficiency through the actual number of matings. The classical counting of semen storage is less suitable for such an analysis since the semen of one drone is sufficient to fill the spermatheca of an *A. mell.* queen (Moritz & Southwick 1992). Tests using matings of *cordovan* queens with *cordovan* and wildtype drones (Taber 1954) gave only vague estimations of the queen mating frequency of the tested colonies.

In this study we use DNA-microsatellite variability to assess the number of matings through the intracolonial relatedness (Estoup *et al* 1994). We compare queen mating frequency on three classical types of mating stations. Island mating yards, pure breed area mating stations and isolated mountain mating yards.

Materials and methods

(a) description and management of the tested mating yards

Worker samples were taken from 27 queenright colonies of *Apis mellifera carnica* (Table I). We examined colonies with queens that were mated on islands (Langeoog and Norderney), on low land mating yards (Gramschatzer-Wald and Königswald) and mountain mating yards (Rachel-Diensthütte and Hochgrat an isolated high mountain station, Table I). All locations are used as commercial mating apiaries in Germany with different numbers of drone colonies ranging from 10-42 (Table I). From each colony worker samples were taken and stored in EtOH in a freezer until further processing.

(b) DNA isolation and microsatellite analysis

DNA was phenol extracted from individual workers following the protocols of Beye and Raeder (1993) with the following changes: 1. Workers were incubated in insect Ringer solution (127 mM NaCl, 1.5 mM $CaCl_2$, 5 mM KCl, pH 7.4 with NaOH) for one hour at room temperature before phenol extraction. 2. Thoraces of individual workers were homogenized in 400 µl of DNA extraction buffer (100 mM NaCl, 100 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.1 % SDS). 3. DNA was resuspended in 30 µl DDH_2O. We used 4 DNA-microsatellite loci in this study. Multiplex PCR of two pairs of loci (A43/B124, A76/A107) was done according to the standard protocols of Estoup *et al* (1993, 1994). Amplification products were electrophorezed on 6% polyacrylamide sequencing gels for 5.5h (A76/A107) or 5h (A43/B124) with M13mp18 control DNA sequencing reactions run on the same gel as size standards. Microsatellite alleles were scored as fragment lengths in base pairs.

c) Genotype analysis and number of observed males

The genotypes of the mother queens and the father drones were determined from the genotypes of the worker samples. The queen was considered to be homozygous if an allele was present in every worker of the colony. The queen was assumed to be heterozygous when every worker carried one of two alleles. The paternal alleles were those not carried by the queen. If approbiate pedigree information was available, we used the putative mother genotype of tested sister queens to exclude additional allele combinations. As a rule we chose the allele combination yielding the lowest number of observed matings (n_o) if multiple queen genotypes were possible at a given locus.

d) Drifted workers

Individuals who did not share one of the queen's alleles at each of the tested loci were considered to be drifted workers and were excluded from further data analysis.

(e) Data analysis

We estimated from the native worker samples the average intracolonial relatedness $\overline{R}$ following Estoup *et al* (1994). Then we calculated the number of effective males (n_e) using the equation of Crozier and Pamilo (1996) solved for n_e:

$$n_e = \frac{2}{4\overline{R}-1}, \qquad (1)$$

where n_e = number of effective males.

For each type of mating yard we estimated differences in queen mating frequency. Using a hierarchical ANOVA model we estimated the variance between the three mating yard types, between yards within and residual variance. Among the lowland and mountain yard types we computed a Mann-Whitney U-test to test for potential differences in queen mating frequency. We calculated a simple correlation (r-matrix, Table I) between the number of observed or effective males and the number of drone colonies at the tested yards. To determine the probability of identical father genotypes we estimated for each yard the product of the highest allele frequency for each locus.

Results

On Langeoog and Norderney 144 paternal genotypes were detected in the 11 tested colonies. The number of observed matings ranged from 6 to 19 (Table II). For the 16 mainland-mated queens we found a range from 11 to 24 patrilines per colony (Table II). The estimates of the effective number of matings ranged from 4.61 to 31.07 (Table II). We found no significant correlation of the number of drone colonies on the number of observed and effective males (Figure 1). We could not observe significant differences of queen mating-frequency among the tested island, among the lowland and between the mountain yards (Figure 2). No differences were found between the tested lowland and mountain yards (Figure 2). Significant lower queen mating frequencies were found for the island mating yards (Figure 2). The allelefrequencies for the identified sexual reproductives at each mating yard are given in table III. The probability of identical father genotypes was lower 2.2% for all yards.

Discussion

The tested 27 *Apis mellifera carnica* queens show a surprisingly high degree of variability in the number of matings. Clearly the worker samples were inadequate to detect rare patrilines in the offspring of our queens (Cornuet & Aries 1980). However this potential error is minor in relation to the extremely high variance for queen mating frequency in our sample. Additional errors such as insufficient microsatellite variability and misinterpretation of drifted workers also seem to be small. The four microsatellites used were able to detect all drone fathers in the offspring of an artificially inseminated queen (Neumann *et al* 1997). However, non identification of patrilines with father drones yielding identical marker combinations strongly depends on allele frequencies. In our sample the probability of identical father genotypes was less than 2.2% due to a high degree of heterozygosity at the used microsatellite loci. Moreover, the level of drifted workers was less than 5% (Neumann & Moritz 1997).

We found strong differences in mating frequency between queens that had mated on islands and on mainland mating yards. What are potential reasons for this phenomenon?

The number of drone colonies at the mating yard does not seem to be the key factor for queen mating frequency since it exceeds the limits discussed by Maul (1972) and Zander & Böttcher (1989). We found no significant correlation of the number of drone colonies at the mainland yards with the queen mating frequency. One might argue that the tested mainland yards differ in the probability of intruding unselected drones from outside the yard that may increase the drone density regardless of the number of drone colonies at the yard. However, also the mating frequencies on the high mountain mating yard Hochgrat (which we believe to be in a drone free environment) were significantly higher than the island results. Moreover, other individual apiary characteristics (Englert 1971, Verbeek 1976, Zander & Böttcher 1989) do not seem to play a major role. We found no significant differences in queen mating frequency between the island or among the mainland mating yards.

Neumann & Moritz. (unpublished data) found that genetic variability among commercial honeybee breeding lines can play a role for queen mating frequency. However, we found no significant differences for queen mating frequencies in our mainland sample although it consists of different breeding lines. Apparently the overall different environmental characteristics of island and continental mating yards are more likely to be responsible for the high differences in queen mating frequency as suggested by Verbeek (1976). Some important features of honeybee mating biology are different on islands. The queen flights were more frequent and shorter than on the continent (Drescher 1965, Englert 1974, Künster 1974, Verbeek 1976). The flight height during mating is distinctly lower than on the continent. Verbeek (1976) found a low drone flight altitude of only a few meters and attempts of copulations at only 1.5 m above ground on the island of Juist. These observations are in line with Ruttner & Ruttner (1963), Bol' Shakova (1978) and Lensky & Demter (1985) who described an influence of climatic conditions on the mating of honeybees. Alber *et al* (1955) showed in their classic experiments on the island of Volcano strong meteorological impacts on honeybee mating behavior. At temperatures below 20° C with overcast sky and wind above 20 miles per hour almost no matings took place on the islands of Volcano. The temperature seems to be an important factor (Alber *et al* 1955, Drescher 1968, Bol' Shakova 1978) which is at the average 2° C cooler on the islands Langeoog and Norderney than on the mainland. Although Meinen (1970) found no impact of wind, Verbeek (1976) claims that wind is the main influencing factor for the mating of honeybees. A wind of up to 7m/sec did not affect the number of drones attracted to the queen in the experiments of Bol' Shakova (1978). The combination of low temperatures (15°-20° C) and high wind velocities (2.6-3.9 m/sec) has been shown to increase the number of short queen flights during which mating does not occur (Lensky & Demter 1985). Wind speeds of more than 5m/sec were assumed to prevent mating itself (Bol' Shakova 1978). Such wind speeds of more than 14 m/sec are frequent on the tested Eastfrisian islands. According to Verbeek (1976) this allows only low-level flight of honeybees leading him to the suggestion that potential drone congregation areas on the Eastfrisian islands could not be used. In general the climatic conditions on the islands which are associated with successful mating (Lensky & Demter 1985) seem to be less favorable than on the mainland (Meinen 1970, Englert 1972). Since queens are most sensitive to changes in weather (Alber *et al* 1955) and climatic conditions do affect queen and drone mating flights (Lensky & Demter 1985) it seems not surprising that this has also an impact on the mating frequency of queens.

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References

Alber, M; Jordan, R; Ruttner, F; Ruttner, H (1955) Von der Paarung der Honigbiene. *Zeitschrift für Bienenforschung* 3 (1), 1-28

Beye, M; Raeder, U (1993) Rapid DNA preparation from bees and % GC fractionation, *Bio Techniques* 14 (3), 372-374

Bol' Shakova, M. (1978) The flight of honey bee drones *Apis mellifera* L. (Hymenoptera, Apidae), to the queen in relation to various ecological factors. *Entomological Review* 56 (2), 53-56

Böttcher FK (1947) Was taugt die Belegstelle? *Der Imkerfreund* 2, 115-116

Böttcher FK (1972) Richtige Führung einer Landbelegstelle. In: Ruttner, F: Ruttner, H; Harnaj, V (eds.) *Paarungskontrolle und Selektion bei der Honigbiene.* Apimondia, Bukarest, Rumania; pp 57-62

Cornuet J-M, Aries F (1980) Number of sex alleles in a sample of honeybee colonies. *Apidologie* 11: 87-93

Crozier RH*;* Pamilo P (1996) *Evolution of social insect colonies*. Oxford University Press, Oxford UK

Drescher W (1974) Betrachtungen zum Thema Belegstellen. *Allgemeine Deutsche Imkerzeitung* (8): 57-62

Englert E (1972) Versuche und Beobachtungen auf der Inselbelegstelle Neuwerk. In: Ruttner, F: Ruttner, H; Harnaj, V (eds.) *Paarungskontrolle und Selektion bei der Honigbiene.* Apimondia, Bukarest, Rumania; pp 49-52

Estoup, A; Solignac, M; Harry, M; Cornuet, JM (1993) Characterisation of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. *Nucleic Acids Research* 21, 1427-1431

Estoup, A; Solignac; M; Cornuet; J.M (1994) Precise assessment of the number of patrilines and genetic relatedness in honeybee colonies. *Proceedings of the Royal Society London* B 258 1-7

Fondrk, MK; Page, RE, Jr.; Hunt, GJ (1993) Paternity analysis of worker honeybees using random amplified polymorphic DNA. *Naturwissenschaften* 80, 226-231

Künster, H (1974) Kritische Bewertung der Belegstellen für Bienenköniginnen unter Berücksichtigung neuer Befunde ihrer Paarungsbiologie. MSc thesis, Rheinische Friedrich-Wilhelms-Universität Bonn

Lensky, Y.; Demter, M. (1985) Mating flights of the queen honeybee (*Apis mellifera*) in a subtropical climate. *Comparative Biochemistry and Physiology* 81a (2), 229-241

Meinen, G (1970) Unter welchen klimatischen Bedingungen finden auf den ostfriesischen Inselbelegstellen Paarungsausflüge statt? *Nordwestdeutsche Imkerzeitung* (22), 207-209

Moritz, RFA; Meusel, M; Haberl M (1991) Oligunucleotide DNA fingerprinting discriminates super and halfsisters in honeybee colonies (*Apis mellifera* L.). *Naturwissenschaften* 78, 98-102

Moritz, RFA; Southwick (1992) *Bees as superorganisms*. Springer Verlag, Stuttgart, FRG

Neumann P; Fondrk K; Page R; Moritz, RFA (1997) Testing the reliability of DNA-microsatellites in instrumental inseminated queen-honeybees (*Apis mellifera* L.). In: Crailsheim, K; Stabenhteiner, A (eds.) *Soziale Insekten, IUSSI-Tagung Graz 1997*, Graz, Austria, p 66, ISBN 3-901864-00-8

Neumann, P; Moritz, RFA (1997) Drifting of workers and drones as an evolutionary factor in the honeybee *Apis mellifera* (L.). In: Proceedings of the sixth congress of the ESEB, Arnhem, The Netherlands

Ruttner, F (1959) Drohnenflugweite und Drohnendichte. *Deutsche Bienenwirtschaft* 10 (1), 42-47

Ruttner, F (1983) *Zuchttechnik und Zuchtauslese bei der Biene*. Ehrenwirth Verlag, München, FRG

Ruttner, F; Ruttner, H (1965) Wie weit fliegen Drohnen und Königinnen? *Bienenvater* 86 (1), 15-21

Ruttner, F; Ruttner, H (1966) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 3. Flugweite und Flugrichtung der Drohnen. *Zeitschrift für Bienenforschung* 8, 332-354

Ruttner, F; Ruttner, H (1972) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 5. Drohnensammelplätze und Paarungsdistanz. *Apidologie* 3, 203-232

Taber, S (1954) The frequency of multiple mating of queen honey bees, *Journal Economical Entomology* 47, 995-998

Tiesler, FK (1972) Die Inselbelegstellen aus dem Norden der BRD. In: Ruttner, F: Ruttner, H; Harnaj, V (eds.) *Paarungskontrolle und Selektion bei der Honigbiene.* Apimondia, Bukarest, Rumania; pp 52-56

Verbeek, B (1976) Untersuchungen der Ausflugsaktivität von jungen Bienenköniginnen unter Festlands- und Inselbedingungen mittels lichtelektronischer Überwachung. *Apidologie* 7 (2), 151-168

Zander, E; Böttcher, FK (1989) *Haltung und Zucht bei der Biene*. Eugen Ulmer Verlag, Stuttgart, FRG

Table I: Examined mating yards with the number of tested colonies and the number of drone colonies at the apiary (N = native worker sample per tested yard).

Yard type	Yard	Colonies	N	Drone colonies
Island	Langeoog	7	290	10
	Norderney	6	196	15
Low land	Gramschatzer-Wald	6	205	20
	Königswald	4	152	42
Mountain	Rachel-Diensthütte	3	100	33
	Hochgrat	3	112	10
Σ	6	29	1055	-

Table II: Number of observed (n_o) and effective males (n_e) of the tested queens at the different types of mating yards (N = sample size of native workers)

Yard type	Yard	Colony	N	n_o	n_e
Island	Langeoog	L2	33	13	12.28
		L3	48	16	13.59
		L4	57	16	15.49
		L5	51	9	8.28
		L6	49	11	9.12
		L7	52	14	11.95
		Σ	290	13.17 ± 1.14	11.79 ± 1.14
	Norderney	N1	35	6	4.61
		N2	41	16	13.23
		N3	43	13	11.01
		N4	25	10	10.71
		N6	52	19	19.79
		Σ	196	12.8 ± 2.27	11.87 ± 2.44
	Islands total	Σ	486	13 ± 1.14	11.82 ± 1.19
Low land	Gramschatzer-Wald	GW1	34	18	18.1
		GW2	31	13	14.09
		GW3	39	11	10.1
		GW4	30	22	31.07
		GW5	32	20	31
		GW6	39	24	22.45
		Σ	205	18 ± 2.08	21.14 ± 3.55
	Königswald	KW1	58	20	17.59
		KW2	38	24	26.04
		KW3	24	12	12
		KW4	32	12	13.05
		Σ	152	17 ± 3	17.17 ± 3.2
Mountain	Hochgrat	HG1	38	20	17.74
		HG2	32	17	20.67
		HG3	42	22	26.91
		Σ	112	19.67 ± 1.45	21.77 ± 2.7
	Rachel-Diensthütte	RD1	35	18	17.5
		RD2	39	21	27.44
		RD3	26	15	21.67
		Σ	100	18 ± 1.73	22.2 ± 2.88
	Mainland total	Σ	569	18.06 ± 1.08	20.46 ± 1.65

Table III: Allelefrequencies (four microsatellite loci: A76, A107, B124 and A43; allele size in base pairs) for the tested mating yards (L = Langeoog; N= Norderney; GW = Gramschatzer-Wald; HG = Hochgrat; KW = Königswald; RD = Rachel-Diensthütte). Only the identified sexual reproductives are considered. The probability P for non detected patrilines over all loci is given.

Locus	N	Alleles																										
A76		209	231	233	239	243	249	251	259	261	265	267	271	277	279	281	283	287	289	291	295	299	305	311	313	325	331	343	353	357
L	103	0	0	0	0	0	0	0	0	0	0.24	0	0.14	0	0	0.08	0.02	0.07	0.08	0.06	0	0.29	0.01	0	0.10	0	0	0	0	0
N	82	0	0.16	0	0	0	0	0	0	0	0.29	0	0	0	0	0.01	0.02	0	0	0.10	0	0	0	0	0	0	0	0.01	0.21	0.20
GW	119	0.01	0.01	0	0.02	0.03	0.06	0.10	0.04	0.03	0.09	0	0.08	0.03	0	0	0.07	0.02	0	0	0	0.08	0.10	0.03	0.12	0	0.01	0.03	0.07	0
HG	63	0	0	0.06	0.02	0.14	0	0.05	0.13	0	0.08	0.14	0.10	0	0.03	0	0.02	0.11	0	0	0	0.06	0.02	0	0.02	0.02	0.02	0	0	0
KW	75	0	0.04	0.03	0.03	0.03	0.04	0	0.04	0	0.01	0.11	0.04	0.01	0.04	0.05	0.01	0.09	0	0	0.09	0.01	0.03	0.04	0.09	0.05	0	0	0.01	0
RD	60	0	0.17	0	0	0.02	0	0.03	0.07	0	0	0	0.03	0.05	0.03	0.03	0.2	0	0	0.07	0.05	0.18	0.03	0	0.02	0	0	0.02	0	0
A107	N	141	158	159	160	162	163	164	165	166	167	168	169	170	171	172	173	174	176	177	181	183	189							
L	103	0.06	0.10	0	0.18	0	0.16	0	0.12	0	0	0	0	0.30	0.01	0	0	0	0.08	0	0	0	0							
N	82	0.11	0	0	0	0	0.10	0	0.18	0	0	0	0	0.34	0	0	0	0	0.27	0	0	0	0							
GW	120	0	0.13	0	0.09	0.11	0	0.06	0	0.01	0	0	0	0.06	0.03	0.11	0	0.08	0.04	0.05	0.05	0.03	0							
HG	65	0	0.05	0.02	0.19	0	0	0	0.03	0.09	0	0.08	0.09	0.14	0.06	0.02	0.03	0.03	0.03	0.08	0	0	0.03							
KW	76	0	0.12	0.01	0.07	0.06	0.08	0.04	0	0.09	0.11	0.11	0	0.03	0.09	0.01	0	0.03	0.10	0	0.01	0.05	0							
RD	60	0	0.08	0	0.12	0.1	0	0.03	0	0.1	0	0.08	0	0.05	0.03	0.02	0	0.02	0.1	0.23	0	0.05	0							
B124	N	212	214	216	218	220	222	224	228	230	232	234																		
L	94	0	0.23	0.40	0.14	0.15	0.05	0	0.02	0	0	0																		
N	82	0	0.35	0.19	0	0	0.23	0	0.23	0	0	0																		
GW	120	0	0.41	0.29	0.13	0.06	0.03	0.02	0.03	0.02	0	0.01																		
HG	65	0.03	0.54	0.279	0.09	0.03	0.02	0	0	0	0.02	0																		
KW	76	0.01	0.64	0.23	0.12	0	0	0	0	0	0	0																		
RD	60	0	0.67	0.07	0.12	0.02	0.02	0	0.02	0.1	0	0																		
A43	N	124	126	127	128	132	139	140	141	142	146				P															
L	102	0	0.02	0.09	0.06	0.03	0	0.62	0	0.18	0				L		0.021													
N	81	0	0	0.03	0.21	0	0.05	0.47	0.03	0.21	0				N		0.017													
GW	119	0	0.19	0.49	0	0	0	0.31	0	0.01	0				GW		0.003													
HG	65	0	0.09	0.37	0	0	0.03	0.48	0	0.03	0				HG		0.007													
KW	76	0	0.11	0.38	0	0	0.01	0.41	0	0.01	0.08				KW		0.004													
RD	60	0.02	0.07	0.42	0	0	0	0.15	0	0	0.35				RD		0.012													

Figure 1: Regression of the number of drone colonies at the tested mating yards on the number of effective males. No significant effect was found (simple correlation [r-matrix], $Rn_o = 0.23$; $P > 0.05$; $Rn_e = 0.28$; $P > 0.05$).

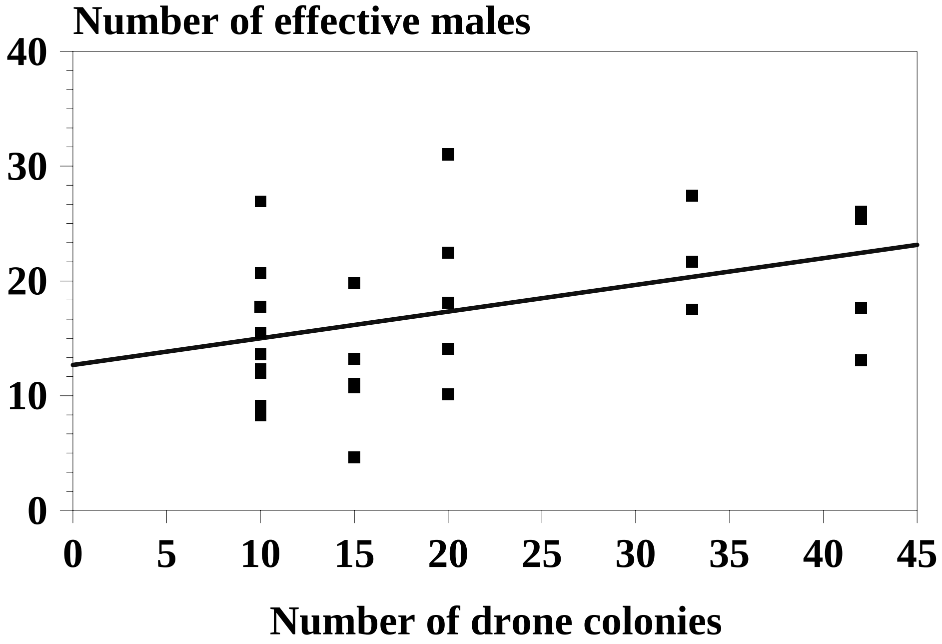
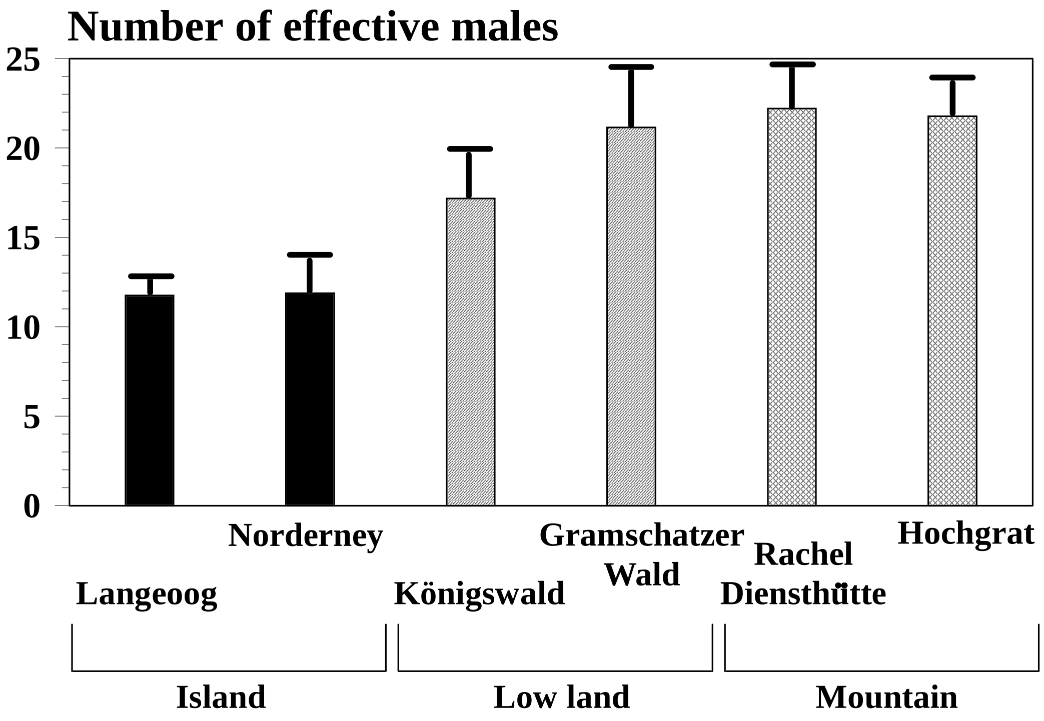


Figure 2: Mean number of effective males on the two islands and on the four mainland mating yards. The mean number of effective males (x ± s.e.) is given. Significant differences among the tested yard types were detected (hierarchical ANOVA model, between yard types: n_o: F= 36.58, P < 0.05; n_e: F= 26.83, P < 0.05). No significant differences between the islands, among the lowland yards and among the mountain yards were found (hierarchical ANOVA model, between yards: n_o: F= 0.12, P > 0.05; n_e: F= 0.35, P > 0.05).



III

Testing the reliability of DNA microsatellites in instrumentally inseminated queen honeybees (*Apis mellifera* L.)

(Zuverlässigkeitstest für DNA-Microsatelliten in instrumentell begatteten Bienenköniginnen [*Apis mellifera* L.])

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DNA-Microsatellites are powerful tools for the genetic analysis of social insect colonies. In recent years many empirical studies used this technique to determine the actual genotypic composition of nests. The reliability of the method has not been rigorously tested. High mutation rates at polymorphic insect microsatellite loci and methodological errors may distort results. Studies based on adult worker samples may overestimate polyandry because of undetected foreign individuals.

In order to test the reliability of DNA microsatellite loci in social insects a virgin honeybee queen was instrumentally inseminated using semen of 10 drones. Sperm were mixed by diluting them in TRIS buffer then centrifugation. Brood combs with emerging adults were placed into an incubator then workers were collected as they emerged. DNA was extracted from 184 workers and the samples were combined with additional 21 DNA samples from unrelated workers. The unrelated workers simulated the effects of „drifting“ resulting from orientation errors. In a double blind test, all samples were genotyped using 4 DNA microsatellite loci. The genotypes of the mother queen and the father drones were determined from the worker genotypes. Workers not sharing one of the queen’s alleles at each locus were considered to be foreign „drifters“. 24 samples yielded incomplete genotype data and were excluded from further analysis. In the remaining 181 individuals all ten patrilines were found with frequencies ranging from 2 to 47. 14 foreign workers within this sample were correctly identified. One foreign worker was not found. Our results show that in 181 meioses no visible mutational events occur indicating that the tested microsatellites are as reliable as human microsatellites used in forensic medicine. Undetected drifting of workers and methodological errors did not effect the results in this study. Such an exact documentation of the intracolonial worker population of honeybees is necessary for studies of evolutionary biology and questions of practical bee management.

IV

Testing the reliability of the honeybee performance yard Schwarzenau using DNA microsatellites

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Problem:

Evaluating performance data of honeybee sister queen colonies can suffer from two short comings caused by undesired group composition: 1. "Sister queen" groups are not composed of sister queens due to mistakes in queen rearing management. 2. Drifting of worker honeybees.

Method:

Honeybee workers (n = 1560) were sampled from 39 colonies belonging to 10 performance groups at the performance yard Schwarzenau (Germany) in June 1995 and genotyped using 4 DNA microsatellite loci. If performance groups were composed of combined queen genotypes with more than 2n + 2 alleles per locus (N = number of queens), they were comprised non-sister queens. Workers who did not share one of the queen's alleles at each of the tested loci were considered as drifted individuals.

Results:

One of the tested breeding lines was identified to consist of unrelated queens. Drifting of workers ranged from 0 to 14% with a mean of 4.63%. Drifters from neighbouring colonies did not prefer each others home colony and no significant effect of the row position was found.

Discussion:

Drifting of workers was distinctly lower in Schwarzenau than in previously studies of commercial apiaries. The low number of drifted workers is unlikely to have an impact on evaluating performance data. However, unrelated queens can seriously distort results but this can be detected using molecular DNA techniques as shown in this report.

V

The impact of polyandry on the phenotype of honeybee (*Apis mellifera* L.) colonies

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Submitted to Behavioral Ecology

Running title: Polyandry and phenotypes of honeybee colonies

Abstract

The phenotypes of thirty queenright honeybee colonies with naturally mated queens were evaluated over a two years period. Colony size, honey yields and colony levels of infestation with *Varroa jacobsoni* were assessed. Worker samples were taken from each tested colony. Individual workers were genotyped at four DNA-microsatellite loci to determine the degree of polyandry. We found significant correlations between colony size and honey yield and between honey yields and colony sizes of two subsequent years. Analyses of covariance revealed a strong impact of the breeding lines on honey production and on infestation with *Varroa jacobsoni*. The impact of polyandry on the tested phenotypic traits was weak and in no case significant but generally had a positive effect on colony productivity. In spite of a large number of plausible theories for the evolution of extreme polyandry in honey bees, it remains difficult to document empirical evidence for fitness advantages at the level of colonial phenotypes clear.

Key words: *Apis mellifera*, colony size, DNA-microsatellite, evolution, genotypic variability, honeybee, honey yield, parasite, polyandry, *Varroa jacobsoni*

Introduction

Multiple mating of females is a widespread phenomenon in eusocial Hymenoptera (Crozier and Pamilo 1996). Especially honeybees show an exceptional high level of polyandry (Boomsma & Ratnieks 1996). Through the development in microsatellite technology (Estoup *et al* 1994) detailed studies have revealed matings of honeybee queens with more than 25 males (Moritz *et al* 1995, Oldroyd *et al* 1996, 1997, Neumann *et al*, 1998a,b). This seems counterintuitive in light of inclusive fitness benefits derived from monandry (Hamilton 1964a,b, Gadagkar 1990) and in light of potential costs of multiple matings of the queen (Moritz *et al* 1995, Oldroyd *et al* 1996, 1997 but see Ratnieks 1990).

Obviously polyandry can only evolve if the fitness benefit exceed the costs of additional matings. The fitness of honeybee colonies is clearly the number of surviving swarms produced (Moritz & Southwick 1992). Colony phenotype characteristics which enhance or reduce the likelihood of producing viable swarms are the cues to colony fitness. These should be enhanced under larger genotypic variability. This could be either in terms of superior performance of more genetic diverse colonies or in terms of a reduced phenotypic variance (Page *et al* 1995) assuming colonies with average phenotypes have a superior chance of reproduction. Many hypotheses have been proposed to explain the evolution of polyandry (Crozier & Page 1985, Crozier & Pamilo 1996, Boomsma & Ratnieks 1996). Most theoretical and empirical studies focused on the genetic variance hypotheses (Pamilo 1993, Keller & Reeve 1994) which predict that colonies gain fitness through a low intracolonial relatedness. Several potential mechanisms have been proposed to explain such an increase in colony fitness (Page 1980, Crozier & Page 1985, Sherman *et al* 1988, Ratnieks 1990).

The parasite and pathogen model predicts that genetic variance reduces the susceptibility of colonies to parasites and pathogens (Hamilton 1987, Sherman *et al* 1988, Hamilton *et al* 1990, Shykoff & Schmid-Hempel 1991a,b). In light of their arguments polyandry in social insects has evolved as an adaptive response to high parasite and pathogen loads similar to the evolution of sex (Maynard Smith 1971). The findings of Shykoff and Schmid-Hempel (1991a,b) for colonies of the bumble-bee *Bombus terrestris* and its parasite *Crithidia bombi* seem to give empirical support to the parasite model although other explanations are also possible.

Alternatively, the input of genetically based task specialization on colonial phenotypes has led to several versions of the division of labor hypothesis for the evolution of polyandry (reviewed by Robinson 1992). Polyandry should ensure a broader variety of genetic specialists (Hunt & Page 1995) and thus allow for a more efficient division of labor through task specialization of individual workers (Crozier & Page 1985, Calderone & Page 1991, Oldroyd *et al* 1992a, Dreller *et al* 1995). Empirical support for this hypothesis was provided by Oldroyd *et al* (1992b) and Fuchs & Schade (1994) who found increased performance of colonies headed by queens with a higher level of polyandry. This may result in increased performance of colonies with higher genotypic diversity or in an reduced phenotypic variance if polyandry stabilizes colony behavior (Crozier & Page 1985). Page *et al* (1995) extended this hypothesis and suggested that colonies with a more genotypic diverse workforce are less likely to fail. They found that a group of colonies with a higher amount of genotypic diversity was average for nearly all phenotypic traits measured relative to groups with a lower genotypic diversity. In light of their results, genotypic diversity increases the probability of expressing an average colony phenotype. Nevertheless, an unambiguous demonstration of the adaptive significance of genotypic diversity at the colonial level is lacking and remains a major problem for explaining the evolution of polyandry (Crozier & Page 1985, Sherman *et al* 1988, Keller & Reeve 1994, Ratnieks & Boomsma 1995).

So, how can we evaluate fitness of honeybee colonies? Even with managed bee colonies it is impossible to assess the number of surviving swarms and the mating success of drones unless entire populations are screened during the whole reproductive season. Colony honey stores can be considered as parameters closely linked to fitness because these stores are essential for surviving periods of dearth. Parasites and diseases can obviously reduce colony fitness. Overall colony size is an impressive characteristic of a social insect colony. Only large colonies have enough workers to produce surviving swarms. Therefore colony growth and size may be closely linked with colony fitness. Moreover, big colonies with a larger work force might per se be more likely to buffer environmental changes than smaller colonies (Crozier & Page 1985). For example defensive behavior seems to increase in relation to group size of honeybees (Southwick & Moritz 1985). Thus, very large groups can be effective at repelling predators at little cost to the colony.

In this study we use the above three parameters to evaluate colony fitness and test the predictions made by the various theories for the evolution of polyandry in the honeybee. For the first time we combine the investigation of colonial phenotypes of naturally mated queens with DNA microsatellite analysis to determine polyandry.

Materials and methods

(a) colony phenotype data

In 1994 and 1995 the phenotypes of 30 queenright *A. mell. carnica* colonies headed by naturally mated queens were determined by routine performance testing at the apiary Schwarzenau, Germany (Bayerische Landesanstalt für Bienenzucht 1995). Annual colony levels of infestation with the ectoparasitic mite *Varroa jacobsoni* were evaluated by counting the total number of dead mites in the hives after each of 3 treatments with the acarizide Perizin (Buren *et al* 1992). Honey yield was determined by weighing honey frames before and after honey extraction and by estimating residual winter honey stores. Colony size was determined by estimating the number of sealed brood cells. Since we wanted to document the impact of polyandry we eliminated effects of the genetic values of the breeding strains on the phenotype by standardizing the data. For each colony and each tested phenotypic trait the difference of the raw data point to the mean of its corresponding breeding line was used for further analysis. The absolute differences between the actual phenotypic values of both years were used to determine the phenotypic variability during the testing period.

(b) DNA isolation and microsatellite analysis

At least 40 workers were sampled from each colony and stored in 75% EtOH until further processing. DNA extraction from individual workers was performed following Beye and Raeder (1993) with the following changes: 1) Workers were incubated in insect Ringer solution (127 mM NaCl, 1.5 mM $CaCl_2$, 5 mM KCl, pH 7.4 with NaOH) for one hour at room temperature before extraction. 2) Thoraces of individual workers were homogenized in 400 µl of DNA extraction buffer (100 mM NaCl, 100 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.1 % SDS). 3. DNA was resuspended in 30 µl DDH_2O. We used 4 DNA-microsatellite loci in this study. Multiplex PCR of two pairs of loci (A43/B124, A76/A107) was done according to the standard protocols of Estoup *et al* (1993, 1994). Amplification products were electrophorezed on standard 6% polyacrylamide sequencing gels with M13mp18 control DNA sequencing reactions run on the same gel as size standards. Microsatellite alleles were scored as fragment lengths in base pairs.

c) Genotype analysis and number of observed matings

The genotypes of the mother queens and the father drones were derived from the genotypes of the worker samples. The queen was considered to be homozygous if one allele was present in every worker of the colony. The queen was assumed to be heterozygous for two alleles if every worker carried one of the two alleles. The paternal alleles were those not carried by the queen. If appropriate pedigree information was available, we used the putative mother genotype of the tested sister queens to exclude additional allele combinations. As a rule, we chose the allele combination yielding the lowest number of observed matings as the most parsimonious result. Individuals which did not share one of the queen's alleles were considered to be drifted workers and were excluded from further data analysis in this study.

(d) Data analysis

Due to finite sample sizes the observed number of subfamilies may severely underestimate the actual number of patrilines. Therefore we estimated the number of patrilines according to Cornuet & Aries (1980):

$$E(k) = k - \left[k - \left(1 - \frac{1}{k} \right)^n \right], \qquad (1)$$

Considering a colony of *k* equally frequent patrilines, from which a sample of *n* workers is taken, we numerically evaluated *k* by substituting *E(k)* with our observed number of matings (k_o) and the sample sizes for *n* (Oldroyd *et al* 1997).

We estimated from the native worker samples the average intracolonial relatedness $\overline{R}$ following Estoup *et al* (1994). Then we calculated the number of effective males (m_e) using the equation of Crozier and Pamilo (1996) solved for m_e:

$$m_e = \frac{2}{4\overline{R} - 1}. \qquad (2)$$

We calculated ANOVAs to screen for strain effects on the honey yield and on colony levels of infestation with *Varroa jacobsoni*. For this analysis we used all tested colonies at the apiary Schwarzenau (N = 89 colonies). We tested for potential correlations between the phenotypic parameters and calculated partial correlations between the phenotypic data (corrected for colony size 1994, 1995 and for worker sample size) and the number of queen matings. Finally, the impact of polyandry, breeding line and yearly variance on the colonial phenotype was estimated using a multivariate ANOVA (factors year [1-2] and breeding line [1-10], covariates *k* = number of estimated matings and m_e = number of effective males). This analysis was performed with the raw data set not standardized for the breeding lines. The statistical analyses were performed using the SPSS© statistic package.

Results

A total number of 1290 native workers was genotyped and assigned to drone fathers. The large genotype data set is not included in the paper but is available on request. The numbers of observed matings (k_o) per queen ranged from 10 to 28 with a mean of 17.7 ± 5.23 (Table 1). The number of estimated matings was higher ranging from 10.41 to 54.55 with a mean of 23.95 ± 11.25 (Table 1). The number of effective matings ranged from 7.52 to 39 with an average of 20.1 ± 8.05 (Table 1). The intracolonial relatedness (min = 0.26, max = 0.32, mean = 0.28 ± 0.01) and the absolute colonial phenotypes are given in Table 1. The mean phenotypic values for the tested breeding lines are given in Table 1a. The breeding stocks differed significantly in their honey yields (ANOVA: $p < 0.001$) and in their levels of infestation with *Varroa jacobsoni* (ANOVA: $p < 0.05$). We did find significant positive correlations between the honey yield 1994 and colony size 1994, between honey yield 1994/1995 and colony size 1995, between colony sizes and honey yields of two subsequent years and between the variability of honey yield and colony size (Table 2). However, there were no significant correlations between the phenotypic variances and the sizes of colonies. Thus larger colonies did not result in more homeostatic phenotype expression. We failed to detect any significant correlation between the number of observed, estimated or effective queen matings and the tested phenotype characteristics of colonies (Table 3). Likewise we did not find any significant correlation between the number of observed, estimated or effective queen matings and the variability of the tested phenotypic traits (Table 3) but we did find a weak, non significant positive effect on colony productivity for the honey yield and for levels of infestation with *Varroa jacobsoni*. The multivariate ANOVA revealed a stronger impact of the factors breeding line and yearly variance on the phenotype but also positive effects of polyandry (Table 4).

Discussion

In light of the congruent predictions made by all evolutionary hypotheses it may seen disappointing that we did not find any significant correlation between the tested phenotypes of honeybee colonies and the level of polyandry of queens. However, colonies with a more genetic diverse workforce did show an increased performance, a higher colony size and a reduced *Varroa jacobsoni* infestation in both test years. The impact was however weak and only a small percentage of the phenotype was determined by the effect of polyandry compared to other sources of variation.

If costs for additional matings of the queen are significant we would have expected dramatic effects at the colony level. So what are we missing that we don't find a significant impact of polyandry on the colonial phenotype?
First, our methods for genotyping might be not precise enough for testing the hypotheses. However, the reliability of the employed DNA microsatellite technique for honeybees (Estoup *et al* 1994) has been artificially tested using a queen which was instrumentally inseminated with the semen of 10 drones (Neumann *et al* 1997). In this double blind test 10 patrilines and no visible mutational events were found. The limited sample sizes per colony might have affected our estimates, as discussed in previous papers (Moritz *et al* 1995, Oldroyd *et al* 1995, 1996, 1997). Small sample sizes result in non sampling errors (Boomsma & Ratnieks 1996) or non detection errors of rare patrilines (Cornuet & Aries 1980). But, the probability for non identification errors of patrilines due to identical father genotypes was lower than 2.2% in our sample as a result of the high degree of heterozygosity of the used microsatellite loci in the tested population (Neumann *et al*, 1998a). This seems to be a minor factor compared to the wide range for queen mating frequency (10-28) in this study.

Alternatively, the method of evaluating the performance data could have lacked precision. However, the large number of tested colonies and the repeated measures reduce the consequences of unlikely weighing and counting errors in the performance test routine. Potential effects resulting from drifting individuals between colonies might interfere with the genotypic composition. However, as we show elsewhere (Neumann & Moritz 1998), drifted workers were identified in the samples and did not show any quantitative impact on evaluating colonial phenotype data. Colony sizes and honey yields are positively correlated in our sample as expected from routine beekeeping experience (Sachs 1964). This is a further indication that the phenotype characteristics were properly evaluated.

Another explanation might be that the tested colonial phenotype characteristics are inappropriate to test colony fitness. *Varroa jacobsoni* may have disadvantages for testing the parasite model:

1. *Varroa jacobsoni* and *Apis mellifera* are not a well established host-parasite-system like *Varroa jacobsoni* and *Apis cerana*. No co.-evolutionary process (Thompson 1994) between host and parasite has been taken place. Thus, a higher resistance through a more genetic diverse worker force may not be detected because there is hardly any resistance at all for once infected colonies. This argument does not hold, since we did find genetic variance for colony levels of infestation among breeding lines.
2. In our study the colonies were treated with acaricides such as Perizin to determine the number of mites per colony. This may mask potential effects of polyandry under natural conditions.
3. The resistance of honeybees to *Varroa jacobsoni* is a result of several different complex mechanisms. Already two behavioral and two physiological mechanisms have been found (Kraus & Page in press). Therefore, there is no gene-for-gene relationship between parasite virulence and host resistance as required by the parasite and pathogen model.

Thus, our findings contrast to Woyciechowski *et al* (1994) and Page *et al* (1995) who compared colony levels of infestation with sacbrood, *Ascosphaera apis* and *Bacillus larvae* (Page *et al* 1995) or *Nosema apis* (Woyciechowski *et al* 1994) of singly inseminated queens versus multiply inseminated queens and found no differences. This may result from colonies headed by instrumentally inseminated queens having different phenotypes than colonies with naturally mated queens. For example Wilde (1989) found that colonies with instrumentally inseminated queens produced significant more brood. In light of our findings, we cannot reject the parasite-pathogen hypothesis (Sherman *et al* 1988) to explain at least partly the unusual, high level of polyandry in honeybees.

Finally, it might well be that the honey yield is an unsuitable character to test colony fitness. Just by chance individual foragers may find rich nectar sources. This and many other random environmental effects may influence the amount of honey stored in colonies and only one factor might be a higher foraging efficiency through a more genetic diverse workforce. Moreover, our queens were from commercial honeybee breeding stock. They were mated on mating yards where only few drone producing colonies are available. So, although we tested naturally mated queens the actual degree of genotypic diversity may be less than under true natural conditions. Furthermore our colonies suffer from the cleptoparasitic beekeepers (Moritz & Southwick 1992) which steal great proportions of honey after the foraging season and return an excess of sugar instead. This may balance potential differences in winter survival. Since we did not measure the fitness of our colonies directly in terms of produced swarms, we basically suffer from the problem of how our observed colony traits correlate with fitness under natural conditions. For example if there is an optimal phenotype for colony size (Allee *et al* 1949) and too large colonies are no good our interpretation based on a linear relationship between size and fitness loose explanatory power. The tested traits may be just "tokens" of fitness (Page *et al* 1995) that are associated in some way with natural colony survival and reproduction but we also may be mislead if fitness and tested trait have no linear relationship.

Our results are in line with the findings of Oldroyd *et al* (1992b) and Fuchs & Schade (1994) who found increased performance of colonies headed by queens with a higher level of polyandry. Fuchs & Schade (1994) used artificial small colonies and their drone fathers are from different breeding lines. This might have exaggerated the effects of polyandry in their experiments due to more genetic diverse offspring than in natural populations. Both studies suffer from the pitfall of instrumentally inseminated queens. Moreover, the queens were inseminated with an unnatural low or high number of drones (Oldroyd *et al* 1992b: 1, 2 or 3 drones; Fuchs & Schade 1994: 1 to 6 or 250 drones).

We found empirical support for an increased colony fitness through genetic determined division of labor. Colonies with higher levels of polyandry did produce more honey. Thus, our results contrast to Woyciechowski and Warakomska (1994) who found no differences between the species diversity of pollen gathered by colonies with artificially reduced worker genetic diversity and those derived from naturally mated queens.

There have been numerous reports of genetic influences on division of labor in honey bee colonies, but the impact of worker genotypic diversity on the level of the colonial phenotype remains unclear. Page *et al* (1995) argue that the averaging effect of genotypic variability on colony phenotypes may have selective advantages, making colonies less likely to "fail" because of inappropriate colony responses to changing environmental conditions. In their experiments, colonies with greater genotypic diversity did not have an increased fitness but had a reduced phenotypic variance. However they used artificial inseminated queens and drones from different breeding strains. This may interfere with the results as discussed above. Moreover, as Page *et al*

(1995) pointed out, they analyzed the effects of worker genotypic diversity on the phenotypes of honey bee colonies during a critical phase of colony development, the "nest initiation" phase. Kolmes *et al* (1989) presented data suggesting that genotypic variability may be important when colonies are under environmental stress. Louveaux (1966) found that homogenous performing sister queen colonies showed distinct phenotypic differences when transferred to a new different environment. We do not find any averaging effect of polyandry in our sample. This may result from our mature, unstressed colonies having different requirements. Thus, high levels of polyandry may have an averaging effect (Page *et al* 1995) only during critical phases of colony development such as the post swarming phase or after changing environmental conditions.

In our study the colonies might have encountered conditions too favorable to reveal the full potential benefit of polyandry. On the other hand, the high genetic variance component suggest that the impact of polyandry is very weak in comparison to the breeding line effect. It might well be that all the queens in our sample show at least the minimum level of polyandry necessary for the production of swarms. If we then measure fitness directly or indirectly via fitness parameters as in this study we suffer from the problem that we cannot find any correlation between fitness and polyandry regardless of how precisely our methods of investigation might be.

Honeybees show a well-established mass congregation mating system (Ruttner & Ruttner 1963, 1965, 1966, 1972). Given that there are only little costs of polyandry in honeybees one might expect only small benefits resulting from high polyandry. These may be difficult to measure under field conditions at the level of a colony's phenotype. It has been assumed that polyandry increases the time of the queen's mating flight and therefore increases the risk of queen loss (Moritz & Southwick 1992). For example, polyandry has been assumed to be costly as a result of high predation rates at drone congregation areas (Oldroyd *et al* 1997), sexually transmitted diseases or harsh weather conditions (Moritz *et al* 1995, Oldroyd *et al* 1996). Indeed, Koeniger *et al* (1994) report on the wasp *Vespa affinis* which may have specialized in the hunting and predation of drones at congregation areas. However, the role of these factors in making polyandry costly has never been rigorously quantified in field tests for any social insect mating system in natural populations. So far, only the risk of honeybee queen losses after mating flights has been estimated. However, the results are highly diverse ranging from 1.04% (Ratnieks 1990) in a forest to 26.4% (Tiesler 1972) on North Sea islands. Neumann *et al* (1998a) found that under island conditions the number of honeybee queen matings can be significantly lower than on the mainland presumably reflecting the higher risks. Moreover, the high frequencies of queen losses have been obtained at normal apiaries where the density of nests and consequently the probability of lost young queens due to drifting between nests after orientation or mating flights may be unnatural high (Ratnieks 1990). Losses due to the introduction of the queens to mating nuclei involved in beekeeping practice may overestimate the findings (Ratnieks 1990, van Praagh personal communication). The current picture of the evolution of extreme polyandry in honeybees remains fuzzy. A paucity to find clear fitness advantages in polyandrous colonies is complemented by an extreme wide range of "queen" mating risk estimation. At the current state we would either expect the cost for polyandry to be extremely low or we have missed a critical piece in the benefits resulting from intracolonial variance. Further studies focusing on the cost aspect may be rewarding to contribute to our understanding of the evolutionary proximate factors selecting for extreme polyandry in honeybees.

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References

Alber M, Jordan R, Ruttner F, Ruttner H (1955) Von der Paarung der Honigbiene, Z Bienenforsch 3, 1-28

Allee WC, Emerson AE, Park O, Park T, Schmidt KP (1949) Principles of animal ecology. Saunders, Philadelphia

Beye M, Raeder U (1993) Rapid DNA preparation from bees and % GC fractionation, Bio Tech 14 (3), 372-374

Bayerische Landesanstalt für Bienenzucht (1995) Leistungsprüfung 1993-1995, Prüfhof Schwarzenau, Abschlußbericht. Bayerische Landesanstalt für Bienenzucht, Erlangen, Germany

Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. Phil Trans R Soc B 331, 947-975

Buren NWM, Mariën AGH, Oudejans RCHM, Velthuis HHW (1992) Perizin an acaricide to combat the mite *Varroa jacobsoni*: its distribution in and influence on the honeybee *Apis mellifera*. Physiol Entomol 17 (3): 288-296

Calderone NW, Page RE (1991) The evolutionary genetics of division of labor in colonies of the honeybee (*Apis mellifera*). Am Nat 138: 69-92

Cornuet JM, Aries F (1980) Number of sex alleles in a sample of honeybee colonies. Apidol 11: 87-93

Crozier RH, Page RE Jr. (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. Behav Ecol Sociobiol 18: 105-115

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford UK

Dreller C, Fondrk K, Page RE (1995) Genetic variability affects the behavior of foragers in a feral honeybee colony. Naturwissenschaften 82: 243-245

Estoup A, Solignac M, Harry M, Cornuet JM (1993) Characterisation of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. Nuc Acid Res 21, 1427-1431

Estoup A, Solignac M, Cornuet JM (1994) Precise assessment of the number of patrilines and genetic relatedness in honeybee colonies. Proc R Soc Lon B 258 1-7

Fuchs S, Schade V (1994) Lower performance in honeybee colonies of uniform paternity. Apidol 25: 151-171

Gadagkar R (1990) The haplodiploidy threshold and social evolution. Curr Sci 59: 374-376

Gary NE (1963) Observations on mating behaviour in the honeybee. J Apic Res 2: 3-13

Hamilton, W. D. 1964a. The genetical evolution of social behaviour, I. *J. Theor. Biol.* 7: 1-16

Hamilton, W. D. 1964b. The genetical evolution of social behaviour, II. *J. Theor. Biol.* 7: 1-17-32

Hamilton, W. D. 1987. Kinship, recognition, disease, and intelligence: constraints of social evolution. In Animal societies: theories and facts ed (Itô Y, Brown JL, Kikkawa J). Tokyo, Japan Science Society Press, 81-102

Hamilton WD, Axelrod R, Tanese R (1990) Sexual reproduction as an adaptation to resist parasites (A review). Proc nat acad sci 87: 3566-3573

Keller L, Reeve HK (1994) Genetic variability, queen number and polyandry in social Hymenoptera. Evolution 48: 694-704

Koeniger N, Koeniger G, Mardan M (1994) Mimicking a honeybee queen? *Vespa affinis* indosinensis Péres 1910 hunts drones of *Apis cerana* F. 1793. Ethology 98: 149-153

Kolmes SA, Winston ML, Ferguesson LA (1989) The division of labor among worker honey bees (Hymenoptera: Apidae): the effects of multiple patrilines. J Kans Entomol Soc 62: 80-95

Kraus B, Page R (1998) Parasites, pathogens and polyandry in social Hymenoptera. Am Nat, in press

Louveaux J (1966) Les modalités l'adaptation des abeilles (*Apis mellifica* L.) au milieu naturelles. Ann Abeille 9(4): 323-350

Maynard Smith J (1971) What use is sex? J Theor Biol 30: 319-335

Moritz RFA (1985) The effects of multiple mating on the worker-queen conflict in *Apis mellifera* L. Behav Ecol Sociobiol 16: 375-377

Moritz RFA, Southwick EE (1992) Bees as superorganisms. Berlin, Springer Verlag

Moritz RFA, 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, Fondrk K, Page RE, Moritz RFA (1997) Testing the reliability of DNA-microsatellites in instrumental inseminated queen-honeybees (*Apis mellifera* L.). In: Soziale Insekten, IUSSI-Tagung Graz 1997, Graz, 1997 (Crailsheim K, Stabenhteiner A ed). Graz, ISBN 3-901864-00-8, 66

Neumann P, Moritz RFA, van Praagh J (1998a) Queen mating-frequency on different types of honeybee mating yards. Submitted to J Apic Res

Neumann P, Kryger P, Moritz RFA (1998b) Variability of queen mating-frequency in *Apis mellifera*. Submitted to Mol Ecol

Neumann P, Moritz RFA (1998) Nestmate recognition and the impact of foreign bees on colonial phenotypes of *Apis mellifera*. Submitted to Behav Ecol Sociobiol

Oldroyd BP, Rinderer TE, Buco SM (1992a) Intra-colonial foraging specialism by honey bees (*Apis mellifera* L.) Behav Ecol Sociobiol 30: 291-295

Oldroyd BP, Rinderer TE, Buco SM (1992b) Effects of intracolonial genetic diversity on honey bee (Hymenoptera: Apidae) colony performance. Ann Entomol Soc Am 85: 335-343

Oldroyd BP, Smolenski AJ, Cornuet J-M, Wongsiri S, Estoup A, Rinderer TE, Crozier RH (1995) Levels of polyandry and intracolonial relationships in *Apis florea*. Behav Ecol Sociobiol 37: 329-335

Oldroyd BP, Smolenski AJ, Cornuet JM, Wongsiri S, Estoup A, Rinderer TE, Crozier RH (1996) Levels of polyandry and intracolonial genetic relationships in *Apis dorsata* (Hymenoptera: Apidae). Ann Entomol Soc Am 89(2): 276-283

Oldroyd PB, Clifton MJ, Wongsiri S, Rinderer TE, Sylvester HA, Crozier RH (1997) Polyandry in the genus *Apis*, particularly *Apis andreniformis*. Behav Ecol Sociobiol 40: 17-26

Page RE Jr. (1980) The evolution of multiple mating behaviour by honey bee queens (*Apis mellifera* L.). Genetics 96: 263-273

Page, RE Jr., Robinson GE, Fondrk MK (1989) Genetic specialists, kin recognition and nepotism in honeybee colonies. Nature 338: 576-9

Page-RE Jr; Robinson GE, Fondrk MK, Nasr ME (1995) Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). Behav Ecol Sociobiol 36(6): 387-396

Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. Heredity 70: 472-480

Queller DC (1993) Worker control of sex ratio and selection for extreme multiple mating by queens. Am Nat 142: 346-351

Ratnieks FLW (1990) The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of removal of diploid males. Behav Ecol Sociobiol 26: 343-348

Ratnieks FLW, Boomsma JJ (1995) Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. Am Nat 145: 969-993

Rinderer T.E., Hellmich R.L., Danka R.G., Collins A.M. (1985) Male reproductive parasitism: A factor in the Africanization of European honey bee populations, Science 228, 1119-1121

Roberts WC (1944) Multiple mating of queenbees proved by progeny and flight tests. Glean Bee-Cult 72: 281-283

Robinson G (1992) Regulation of division of labor in insect colonies. Annu Rev Entomol 37: 637-665

Ruttner F, Ruttner H (1963) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 1. Proc 19th Int Congr Apicult, Prague, Apimondia, 604-605

Ruttner F, Ruttner H (1965) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 2. Beobachtungen an Drohnensammelplätzen. Z Bienenforschung 8: 1-8

Ruttner F, Ruttner H (1966) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 3. Flugweite und Flugrichtung der Drohnen. Z Bienenforschung 8: 332-354

Ruttner F, Ruttner H (1972) Untersuchungen über die Flugaktivität und das Paarungsverhalten der Drohnen. 5. Drohnensammelplätze und Paarungsdistanz. Apidol 3: 203-232

Sachs HG (1964) Vergleich der Honigerträge von Bienenvölkern der Carnica-Rasse bei unterschiedlicher Raumumgebung und unterschiedlicher Volksstärke. Z Bienenforsch 7 (2): 47-52

Sherman PW, Seeley TD, Reeve HK (1988). Parasites, pathogens and polyandry in social Hymenoptera. Am Nat 131: 602-10

Shykoff JA, Schmid-Hempel P (1991a) Parasites and the advantage of genetic variability within social insect colonies. Proc R Soc Lon Ser B 243: 55-8

Shykoff JA, Schmid-Hempel P (1991b) Genetic relatedness and parasite-mediated selection on the genetic composition of groups. Behav Ecol Sociobiol 28: 371-6

Southwick EE, Moritz RFA (1985) Metabolic response to alarm pheromone in honeybees. J Insect Physiol 31: 389-392

Thompson JN (1994) The coevolutionary process. London, The University of Chicago Press

Tiesler FK (1972) Die Inselbelegstellen aus dem Norden der BRD. In: Paarungskontrolle und Selektion bei der Honigbiene (Ruttner F, Ruttner H, Harnaj V ed). Bukarest, Apimondia

Wilde J (1989) Development and productivity of honeybee colonies with naturally and artificially inseminated queens. In: Proceedings of the XXXIst International Congress of Apiculture, Warsaw, Poland, August 19-25, 1987. Bucharest, Rumania; Apimondia Publishing House 442-444

Woyciechowski M, Warakomska Z (1994) Worker's genetic diversity has no relation to pollen diversity in a honey bee colony (*Apis mellifera* L.). J Ethology 12(2): 163-167

Woyciechowski M, Król R, Figurny E, Stachowicz M, Tracz M (1994) Genetic diversity of workers and infection by the parasite *Nosema apis* in honey bee colonies (*Apis mellifera*). In: Eds. 12th Congr of the International Union for the study of social insects IUSSI, Paris, 1994 (Nenoir A, Arnold G, Lepage M ed). Paris, Université Paris Nord, 347

Table 1: Genotype and phenotype data of the tested *A. mell. carnica* colonies. The number of observed (n_o), estimated subfamilies *k*, effective males (n_e), the worker sample sizes (N) and the colonial phenotypes for the years 1994 and 1995 are given. All colonies were headed by the same queens during the two-years testing period (BL = breeding line, R = average intracoloníal relatedness as defined by Estoup *et al* 1994).

Colony	BL	n_o	*k*	n_e	R	N	*Varroa* infestation [number of mites]		Honey yield [kg]		Colony size [brood frames]	
							1994	1995	1994	1995	1994	1995
1	A	18	23.18	18.1	0.28	34	161	286	34.6	40.5	6	8
2	A	11	11.31	10.10	0.3	39	154	311	29.4	30.3	6	7
3	B	18	22.69	17.5	0.28	35	520	1355	38	32.5	7	7.5
4	B	21	27.47	27.44	0.27	39	385	402	37.7	33.4	9	7.75
5	B	15	20.75	21.67	0.27	26	472	206	34.8	46.1	6.5	7
6	C	20	21.32	17.59	0.28	58	375	432	49.3	41.7	9	9
7	C	14	14.73	14.13	0.28	24	399	951	32.8	33.7	5.5	6
8	D	20	25.68	17.74	0.28	38	320	512	27.7	38.3	5.5	7
9	D	17	21.93	20.67	0.27	32	393	95	26.1	51	4	8
10	E	11	12.83	9.52	0.3	24	234	378	57.3	43.5	8	9
11	E	21	21.93	19.84	0.28	32	173	388	44.6	42.6	6.5	8
12	E	18	21.54	14.96	0.28	38	182	718	44.4	16.5	8	6
13	F	13	14.63	14.09	0.29	31	231	631	40.2	38.4	7	8
14	F	22	33.05	31.07	0.27	30	283	559	49.5	27.3	7	6
15	F	20	30.05	31	0.27	32	268	492	56.1	34.5	8	8
16	F	25	15.31	15.32	0.28	38	301	326	34.8	41.4	7	8
18	G	28	34.8	29.06	0.27	56	149	952	54.1	55.6	8	9.5
19	G	26	54.55	33.06	0.27	35	186	1050	54.2	47.4	8	10
20	G	17	23.17	12.79	0.29	30	149	110	50.4	40.8	9	9
21	G	20	24.04	14.84	0.28	42	178	175	33.4	34.6	6.25	7.5
22	H	10	10.41	9.36	0.3	32	319	417	56.5	53.4	8	10
23	H	11	25.66	28.88	0.27	22	285	263	20.2	38.9	7	6.5
24	H	18	27.14	25.38	0.27	29	446	705	41.6	52.6	7	10
25	I	14	17.24	18.9	0.28	28	324	426	54.5	41.6	8	8.5
26	I	15	20.75	23.1	0.27	22	160	1039	53.7	44	9	9
27	I	14	18.18	19.12	0.28	26	322	375	46.8	41.7	7	7.5
27	I	26	51.04	28	0.27	36	398	255	54.4	46.3	9	9.5
28	J	10	10.41	7.52	0.32	32	358	1753	53.8	54	10	10
29	J	12	13.82	10.64	0.3	27	188	525	45.2	43.5	7	8
30	J	26	48.63	39	0.26	39	188	249	59.9	55.4	7	9.5

Table 2: Correlation matrix for the performance data of the tested honeybee colonies in the years 1994 and 1995. Colony size, colony levels of infestation with *Varroa jacobsoni*, honey yield of colonies and variance for the tested phenotypic traits during the two-years testing period were considered. Significant correlations are indicated with * for p < 0.05, ** for p < 0.01 and * for p < 0.001.**

		Colony size		*Varroa* infestation		Honey yield		Phenotypic variance		
		1994	1995	1994	1995	1994	1995	Honey	Size	Varroa
Colony	1994	1								
Size	1995	0.50**	1							
Varroa	1994	-0.01	0.25	1						
Infestation	1995	0.27	0.15	0.23	1					
Honey	1994	0.51**	0.70***	0.08	0.14	1				
Yield	1995	0.06	0.77***	0.21	-0.03	0.44*	1			
Phenotypic	Honey	-0.08	-0.23	-0.09	-0.11	-0.12	-0.10	1		
Variance	Size	0.14	-0.18	-0.07	0.06	-0.16	-0.23	0.36*	1	
	Varroa	0.17	0.13	0.08	0.33	-0.03	0.06	-0.36*	-0.03	1

Table 3: Correlation matrix (simple correlations, r-matrix) between colony size 1994 and 1995 and the number of estimated matings (*k*) and effective males (m_e). Partial correlations (corrected for colony size 1994 and 1995 and worker sample size) between the number of estimated (*k*) and effective males (m_e) and the performance data of the tested honeybee colonies in the years 1994 and 1995. Colony levels of infestation with *Varroa jacobsoni*, honey yield of colonies and phenotypic variance during the two-years testing period were considered. No significant correlations are found.

		k	m_e
Colony size	1994	-0.06	-0.06
	1995	0.09	0.03
Varroa infestation	1994	0.01	-0.20
	1995	0.00	-0.11
Honey yield	1994	0.21	0.25
	1995	-0.07	0.10
Phenotypic variance	Honey	-0.14	0.05
	Size	0.18	0.25
	Varroa	0.19	0.07

Table 4: Results of the multivariate ANOVA for the impact of the breeding line, of the year and of polyandry on the colonial phenotype (factors year [1-2] and breeding line [1-10]). The regression analysis was calculated for both covariates (k = number of estimated matings and number of m_e = effective males). The analysis was performed with the raw data set not standardized for the breeding lines (B = regression coefficient, C_{lower} / C_{upper} = ± 95% confidence limits).

Covariate *k*

ANOVA

Variable	Year by Breeding line		Breeding line		Year	
Phenotype 1994/1995	F-value	P	F-value	P	F-value	P
Honey yield	2.01	0.064	2.13	0.05	0.05	0.832
Colony Size	0.73	0.683	2.03	0.06	8.49	0.006
Varroa infestation	0.77	0.646	0.98	0.47	8.17	0.007

Regression analysis

Dependent variable Phenotype 1994/1995	B	C_{lower}	C_{upper}	t-value	P
Honey yield	0.1498	-0.076	0.376	1.341	0.188
Colony Size	0.0186	-0.013	0.050	1.184	0.244
Varroa infestation	-3.3818	-11.138	4.374	-0.882	0.383

Covariate m_e

ANOVA

Variable	Year by Breeding line		Breeding line		Year	
Phenotype 1994/1995	F-value	P	F-value	P	F-value	P
Honey yield	2.01	0.064	2.10	0.054	0.454	0.832
Colony Size	0.73	0.683	2.14	0.049	8.25	0.007
Varroa infestation	0.77	0.646	0.92	0.520	8.32	0.006

Regression analysis

Dependent variable Phenotype 1994/1995	B	$\mathbf{C_{lower}}$	C_{upper}	t-value	P
Honey yield	0.1762	-0.124	0.476	1.187	0.242
Colony Size	0.0112	-0.031	0.054	0.532	0.597
Varroa infestation	-6.617	-16.328	3.996	-1.227	0.227

VI

Nestmate recognition and the impact of foreign bees on colonial phenotypes of *Apis mellifera*

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Abstract

The presence and origin of foreign workers and drones in honey bee colonies, the impact of foreign bees on the host colony's phenotype and the effects of polyandry on the amount of foreign bees were studied in *Apis mellifera* L. Colony size, honey yield and colony levels of infestation with the ectoparasitic mite *Varroa jacobsoni* were evaluated. Individuals (n = 1359 workers, n = 449 drones) were genotyped using four DNA microsatellite loci. Bees who did not share one of the host queen's alleles at each tested locus were classified as foreign, drifted individuals. The number of effective queen matings was derived from the native worker offspring. The proportion of foreign individuals in a host colony was defined as immigration. Colonies were identified as putative mother colonies if a queen's genotype corresponds with the genotype of a drifted individual. The presence of mother colony members in foreign host colonies was defined as emigration. Drones emigrate and immigrate significant more frequently than workers. Although the immigration and emigration of drones was significantly correlated, this was not for workers. This indicates a caste specific adoption/rejection mechanism. The impact of foreign bees on the phenotypes of host colonies was weak and in no case significant. Likewise, the number of effective queen matings had no or only weak effects on the amount of foreign bees in colonies. We conclude that the foreign label rejection model is unlikely to explain our findings. But we were not able to discriminate between the Gestalt and the common label acceptance model.

Running title: Foreign bees, colonial phenotypes and nestmate recognition in *Apis mellifera*

Keywords: *Apis mellifera*, colony size, drifting, honey yield, microsatellites, nestmate recognition, parasite, phenotype, polyandry

Introduction

In spite of conflicts within colonies, the colony is a basic functional unit in eusocial insects (Moritz & Southwick 1992). The co-operative efforts of colony members positively affect reproductive output and colony maintenance (Crozier & Pamilo 1996). Inclusive fitness theory (Hamilton 1964a,b) predicts particularly strong selective advantages for societies in which the members are closely related. Thus, social insect colonies should be societies which colony members carefully scrutinize nest mates and non nest mates resulting in nestmate recognition as a cornerstone of colony integration (Crozier & Pamilo 1996). Especially for honeybees there is a detailed evidence for a well developed nestmate recognition system (Breed 1983, Breed & Julian 1992, Breed et al 1992 among others).

Nevertheless it is long known that in spite of the guard bees which scrutinize incoming bees and reject non-nest members (Butler & Free 1952, Breed et al 1992), drones and workers from foreign colonies can enter the hive and are adopted as new nest members (Rauschmayer 1928, Butler 1939). The guard bees apparently discriminate between robbing bees which are in search of food and lost bees which offer food to the guards in order to be accepted in the colony.

This adoption of new foreign nestmates has been coined drifting (Rauschmayer 1928, Butler 1939) and requires two mechanisms: 1) individuals get “lost“ as a result of orientation errors and 2) the individuals are adopted as new nest members by the workforce of the host colony. The proximate reasons for the probability and kind of orientation errors of drones and workers have been studied in detail and seem to depend on a variety of factors (Free 1958, Free and Spencer Booth 1961, Jay 1965, 1966a,b, 1968, 1969a, 1971, Vollbehr 1975, Currie 1982, Currie 1986, Currie & Jay 1988, Moritz & Neumann 1996 among others). The arrangement of colonies in apiaries seems to be one important factor (Jay 1966a, 1968, Moritz & Neumann 1996), e.g. there is particularly strong drifting of foreign workers (Free and Spencer Booth 1961, Jay 1965, 1966a,b, 1968) and drones (Moritz & Neumann 1996) into colonies which were placed at the end of a row.

Beekeepers report that drones can freely drift between colonies and are readily accepted in neighboring colonies. It seems as if drones very easily gain admittance into foreign colonies (Washington 1967). Detailed studies yield mixed results. Whereas some authors find drones to drift more frequently than workers (Goetze 1954, Free 1958, Witherell 1965) other claim that drones drift as much as workers (Butler 1939, Livenetz 1952, Lecômte 1958, Kepena 1963). Our studies using multi-locus DNA fingerprinting (Moritz & Neumann 1996) revealed that drifting of drones follows the same underlying behavioral mechanisms as in workers. This seems to be supported by the findings of Giray & Robinson (1996) that behavioral development in drones is regulated by similar mechanisms as in workers.

Numerous predictions and assumptions have been made addressing the potential effects of drifting on the phenotype of honeybee colonies (Lecômte 1958, Jay 1968, Poltev 1968, Di Jong et al 1982, Matheson 1984, Currie 1987 among others). In contrast, there are few papers which try to evaluate the phenotypic impact of drifting, if any, in an experimental setup. Moreover, these detailed studies yield contradictionary results. Whereas some authors found that drifting has for example an impact on the honey yield or on foraging efficiency (Nekrasov 1949, Robinson 1979) others do not find influences (Matvijenko 1965, Jay 1969b, Jay & Dixon 1988). Likewise, the potential role of workers and drones as vectors of various parasites and pathogens (Moreaux 1953, 1959, Lecômte 1958, Jay 1968, Poltev 1968, Di Jong et al 1982, Matheson 1984, Currie 1987) has been shown for the pythopathogenic BBLMV virus (Boyland-Pett et al 1991), for *Bacillus larvae* spores (Goodwin et al 1993) or *Tropilaelaps clareae* mites (Rath et al 1991). Observations of drifting of 600m (Boyland-Pett et al 1991) or 800m (Duranville et al 1991, Mossadegh 1993) indicate that diseases may be spread through between apiaries and whole populations by drifting. But the impact of drifting on the colonial phenotype level remains fuzzy. Goodwin et al (1994) found only a weak impact of drifting on the spread of American foulbrood disease whereas Sakofski (1991) found an interaction between drift and colony levels of infestation with the ectoparasitic mite *Varroa jacobsoni*.

Besides the potential effects of population shifts it remains particular unclear whether balanced levels of drift between colonies which can increase the genotypic diversity of the workforce have an impact or not. Such an increased intracolonial genotypic diversity of colonies has been claimed to enhance colony efficiency. The so-called genetic variability hypotheses try to explain the evolution of polyandry and/or polygyny (Crozier & Page 1985, Keller & Reeve 1994, Page et al 1995).

Since disease loads and honey yields can be regarded as fitness parameters of honeybee colonies (Neumann & Moritz, in review), one would expect effects of drifting at the colonial phenotype level if drifting is relevant for fitness. But are they differences between the impact of drifting workers and drones? Besides the shared effects of potential disease transmission, the adoption of new worker nestmates certainly has a different quality from the adoption of foreign male sexuals. Adopted new worker nestmates increase the work force of the host colony. These additional workers support the native queen's offspring and increase the number of sexual reproductives of the host colony.

On the other hand, foreign laying workers may potentially produce male sexuals, decreasing the host colony's fitness. This effect seems to be minor due to worker policing (Ratnieks & Visscher 1989).

Successful reproduction of laying workers in queenright honeybee colonies is extremely rare but not impossible (Oldroyd et al 1994).

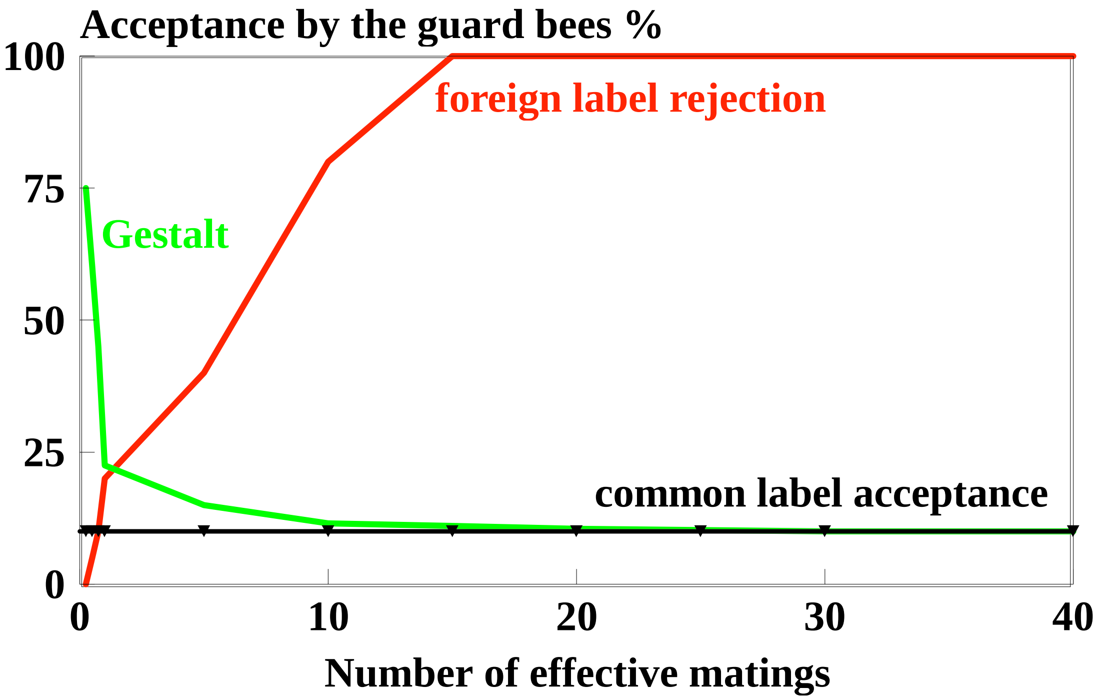
In the absence of a nectar flow, robbing behavior between honeybee colonies may take place, especially when colonies differ in their defence potential. Clearly, robbing workers should be excluded even at high costs because robbing is a real danger for colonies. From an evolutionary perspective it seems therefore plausible that an efficient guarding mechanism should have been evolved which allows the guards to discriminate drifted and robbing bees. Indeed, a drifted worker usually tries to get access to the colony by offering a droplet of food to the guard bees (Winston 1987) to become a fully integrated nest member after acceptance.

In contrast, it seems particularly plausible that guard workers deny access of foreign drones to the colony. Foreign drones do not forage and instead they parasitize on pollen and nectar stores. Moreover, Rinderer *et al.* (1985) found that the presence of drones limits the production of new male sexuals by the host colony (but see Henderson 1994). This has been coined "male reproductive parasitism" by Rinderer *et al.* (1985) and they argued that the drifting of drones had an important impact on the rapid spread of the Africanized honeybee in the Americas. Given, male reproductive parasitism is a major selective force, natural selection should strongly favor nestmate recognition and effective rejection mechanisms for foreign drones. On the other hand colonies which efficiently distribute their drones in other nests also should have a selective advantage because they can produce more males. Consequently the cost of hosting foreign drones for the colony seems to be threefold: a loss of food resources, a decreased production of sexual reproductives through male reproductive parasitism (Rinderer *et al.* 1985) and the potential risk of being infested by parasites and pathogens. Thus depending on the risk of being killed by entering a foreign hive, drifting of drones and efficient recognition/rejection mechanisms should or should not be favored by natural selection.

It was impossible to answer the question if the intracolonial relatedness has an influence on the adoption of foreign individuals in honeybee colonies until the development of appropriate genetic markers. The adoption of new nestmates in honeybee colonies is caused by nestmate recognition errors of the guard bees. Thus, factors which have been assumed to affect nestmate recognition can be important for the amount of foreign individuals in honeybee colonies.

There is evidence for colony recognition in many species (Crozier & Pamilo 1996). The environment can contribute to the colony odor (Stuart 1987, 1988b), but it has been also shown that genetic determinants are highly important (reviewed by Waldmann et al 1988). Genetic variation in kin recognition cues has been demonstrated for bees (Greenberg 1979). In light of the conceptual framework of innate components for colony odor as labels, templates and referents (Lacy & Sherman 1983) two possibilities for the formation of templates may be distinguished (Crozier & Dix 1979): 1) The Gestalt model predicts that individual genotypes are not distinguished but rather a colony odor blend which is formed by the individual labels and their proportions within the social group and 2) The family of individualistic models hypothesizes that individual genotypes are distinguished. This can potentially be the acceptance of individuals bearing exactly the same genotype (genotypic identity), the rejection of individuals possessing labels which are not included in the colony template (foreign label rejection) or the acceptance of individuals possessing a label included in the colony template (common label acceptance). In light of observations that monogynous ant species tend to be more aggressive against intruding individuals of their own specie than polygynous ones (Hölldobler & Wilson 1990, Stuart 1991, but see Stuart 1993) lower intracolonial relatedness may reduce nestmate recognition efficiency because the genetically derived odor cues may be more complex in highly polyandrous colonies. Honeybee colonies headed by naturally mated queens show an exceptional high number of patrilines and a high variance for intracolonial relatedness (Neumann et al, in review, in review, Neumann & Moritz, in review). Consequently, honeybees offer a prime system for testing the nestmate recognition models. Under the assumptions of the divergent models we expect different correlations between the adoption of drifted individuals by the guards and the number of effective queen matings (Figure 1):

Figure 1 Expected correlations between the adoption of intruding foreign individuals by the guards and the number of effective queen matings under the predictions of the different nestmate recognition models.



1) Gestalt model

We hypothesize that the higher the number of queen matings the more detailed becomes the odor blend of the colony. Thus we expect a negative correlation between the number of matings and the adoption by the guards.

2) Genotypic identity: We consider this model to be unlikely for honeybees because in a highly polyandrous colony exactly matching genotypes, which are necessary to ensure colony closure, are a rare event.

3) Foreign label rejection:

We assume that the higher the number of queen matings, the more likely is that the labels of the intruding individual are already present in the template of the colony. Consequently, this model predicts a positive correlation between the number of matings and the amount of adopted individuals in honeybee colonies.

4) Common label acceptance:

Under the assumptions of this model, the common label must be derived from the queen to ensure nestmate recognition in a highly polyandrous honeybee colony. Thus, the ability of the guards to discriminate nestmates from non-nestmates is depend upon the frequency of the queen's alleles at the loci encoding for recognition labels. We hypothesize that under this individualistic model the number of adopted individuals is independent from the number of queen matings.

So far, drifting has mostly been studied using body coloration of bees (Butler 1939, Witherell 1965 among others), racial characteristics (Ruttner 1992) or radioactive isotopes (Thyri 1973). Few studies have addressed this question using DNA (Moritz & Neumann 1996) or biochemical markers (Hung & Rubik 1992). The latter techniques have important advantages for evaluating the amount of drifted individuals. No artificial dyes or racial characteristics which may interfere with the behavior are involved. Even if the position of potential mother colonies is not known, as it might be the case for natural occurring nests, the genotypic composition of a host colony's intracolonial drone and worker population can be determined.

In this paper we study the frequency and origin of foreign workers and drones in honeybee (*Apis mellifera* L.) colonies, the effects of polyandry on the amount of foreign individuals in colonies and the impact of foreign bees on the level of a host colony's phenotype. For that purpose we use DNA microsatellites to: 1) genotype queens of potential host and mother colonies, 2) detect foreign drones and workers within host colonies and 3) find out the potential mother colonies of the drifted individuals. This allows to design a drifting network between the tested colonies in a non-experimental setup. Then, we quantify the impact of foreign individuals on the level of a host colony's phenotype. Finally, we test our predictions for the different nestmate recognition models in the honeybee by correlating the emigration and immigration data of workers and drones with the number of estimated and effective queen matings.

Materials and Methods

Experimental design and sampling

38 queenright colonies of *Apis mellifera carnica* were sampled on the performance testing apiary Schwarzenau, Germany (Figure 2). Only queenright colonies were considered to prevent the influence of a colony's queen state on drifting (Currie & Jay 1988, Currie & Jay 1991). Using the special arrangement of hives at Schwarzenau (Figure 2) we tried to minimize known apiary layout effects on the drifting of workers and drones (Jay 1965, 66a,b, 1968, 1969a, Currie & Jay 1988, Moritz & Neumann 1996). The row position of the colonies in the apiary was evaluated as defined by Moritz & Neumann (1996) to estimate whether the effects of apiary layout on drifting (Jay 1965, 66a,b, 1968) are minimized or not. At least 40 sexually mature drones and 40 adult workers were sampled from the outer frames of each colony (drones: n= 14 colonies; workers n= 38 colonies) at the 08.06.1995. The colonies were sampled early in the morning from 6.00 a.m. to 9.00 a.m. before normal drone flight activity begins (Kurrenoi 1954, Oertel 1956, Ruttner 1966). The samples were immediately placed in 75% ethanol and kept at -40° until DNA extraction.

Performance data of colonies

In 1994 and 1995 the phenotypes of 30 queenright colonies belonging to 5 different breeding lines were determined by performance testing at apiary (Bayerische Landesanstalt für Bienenzucht 1995, Neumann & Moritz, in review). Annual colony levels of infestation with the ectoparasitic mite *Varroa jacobsoni* were determined by counting the total number of dead mites in the hive after each of 3 treatments with the acaricide Perizin (van Buren et al 1992). Honey yield was evaluated by weighing honey frames before and after honey extraction and by estimating residual winter honey stores. Colony sizes were estimated using the amount of sealed worker brood frames. In order to eliminate breeding line effects we standardized the phenotype data. For each colony the absolute difference was calculated to the mean of its own breeding line for every trait.

DNA isolation and microsatellite analysis

DNA was phenol extracted from single individuals following routine protocols (Beye & Raeder 1993) with the following changes:

1. Individuals were incubated in insect Ringer solution (127 mM NaCl, 1.5 mM $CaCl_2$, 5 mM KCl, pH 7.4 with NaOH) for one hour at room temperature before phenol extraction.
2. Thoraces of individual drones and workers were homogenized in 400 µl of DNA extraction buffer (100 mM NaCl, 100 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.1 % SDS).
3. DNA was resuspended in 30 µl (workers) or 50 µl (drones) DDH_2O.

We used DNA-microsatellites which were developed by Estoup *et al* (1993) for *Apis mellifera*. Multiplex PCR was done using two pairs of loci (A43-B124, A76-A107) and the protocols of Estoup *et al* (1993, 1994). Amplification products were electrophorezed on standard 6% polyacrylamide sequencing gels with M13mp18 control DNA sequencing reactions run on the same gel as size standards. Microsatellite alleles were scored as fragment lengths in base pairs.

Genotype analysis

The genotypes of the mother queens and the father drones were derived from the genotypes of the sampled workers (Estoup et al 1994, Neumann et al, in review). Since sister queens originated from the same mother we also used this pedigree information to determine the queens' genotypes. If a worker or a drone had no allele in common with the putative mother queen genotype at one or more of the tested loci, the individual was considered to be a drifted individual.

Number of observed, estimated and effective queen matings

The number of observed, estimated and effective queen matings was derived from the worker offspring (Neumann et al, in review, in review, Neumann & Moritz, in review) using the intracolonial relatedness as defined by Estoup et al (1994) and the equation of Crozier & Pamilo (1996). The number of estimated queen matings as defined by Cornuet & Aries (1980) was numerically determined.

Putative mother colonies of drifted individuals

The genotypes of the drifted individuals were assigned to queen genotypes (Neumann et al, in review, in review, Neumann & Moritz, in review, Table 1). If the genotype of a drifted drone corresponds to a potential gamete type of a queen he was considered to be a son of that queen. Drones were excluded from further analysis if a drone's genotype corresponds to more than one queen genotype. In case the genotype of a drifted worker corresponds to a queen genotype she was considered to be an offspring of that queen. If the genotype corresponds to more than one queen genotype, the workers genotypes were compared to the drone fathers of the potential mother colonies. In case no corresponding patriline exist in the potential mother colonies, the possible allele combinations of undetected drone fathers were derived from the allele frequencies of the mating yard where the queens were mated. If no corresponding genotype was detected, the drifted individual was excluded from further data analysis.

Emigration

The relative proportion of a mother colony's individuals (n_i) of the total drifted individuals in foreign host colonies (Σn_i) was defined as the emigration (e_i) of a tested mother colony:

$$e_i = \frac{n_i}{\sum n_i} \qquad (1)$$

Individuals which did not show one of the host queen's alleles at each of the tested loci were considered to be native nest members. Non detected drifted individuals may cause errors. This non-detection error of drifted individuals is the probability that a drifted bee is genetically indistinguishable from the offspring of the host queen. This depends on the frequencies of the host queen's alleles at each of the tested microsatellite loci. In order to estimate this non-detection error, we calculated with the queen allele frequencies of the tested honeybee population the product of the highest queen allele frequencies for each microsatellite locus in case of the drones and the product of the sums of the two highest queen allele frequencies for each locus in case of the workers.

Immigration

The relative proportion of drifted individuals in the sample was defined as the immigration into a host colony.

Data analysis

We compared the immigration and emigration of workers and drones using Mann-Whitney U-tests. We defined the acceptance of a colony as the immigration divided through the emigration and compared the two slopes for drones and workers. We calculated simple correlations (r-matrix) between immigration and emigration of workers and drones with the row position, the number of effective queen matings and the colony size (Bayerische Landesanstalt für Bienenzucht 1995, Neumann & Moritz, in review). Partial correlations (corrected for colony size 1994 and 1995) were performed between the drift and the performance data (Bayerische Landesanstalt für Bienenzucht 1995, Neumann & Moritz, in review) because colony size has been shown to interfere with the honey yield (Sachs 1964) and with the population dynamics of *V. jacobsoni* (Schmidt 1995). The statistical calculations were done using the SPSS© statistic package.

Results

From 1808 genotyped individuals (449 drones and 1359 workers) 65 drifted workers and 236 drifted drones could be assigned to mother colonies (Tables 2-3). The high amount of genotype data for the native individuals is not included in this paper but is available up on request. The emigration of workers ranged from 0 to 15.22 with a mean of 4.63 ± 0.66 (Table 4). For the immigration of workers we found a range from 0 to 14.29 with an average of 4.66 ± 0.69 (Table 4). The amount of emigrated drones reached from 17.07 to 85.29 with a mean of 42.85 ± 6.07 (Table 4). We found an immigration of drones ranging from 2.56 to 89.13 with an average of 50.21 ± 6.80 (Table 4). The non detection error of drifted individuals as estimated from the allele frequencies of the tested honeybee population (Table 5) was $p = 0.0043$ for drones and $p = 0.0194$ for workers. Both immigration and emigration was significantly higher in drones than in workers (Figure 3). We found a significant correlation between the emigration and immigration in drones ($r = 0.66$, $p < 0.02$) which was lacking in the worker sample (Figure 3). The acceptance slope of the drones was significantly higher than for workers. There was neither a significant correlation between drone and worker immigration nor emigration (Table 6). Likewise, no significant correlations of immigration and emigration of workers and drones were observed with the number of estimated queen matings, the number of effective males, the performance data of colonies and the row position (Table 6).
Evidence for Gestalt common label acceptance foreign label rejection?

Discussion

Drifting of honeybees clearly is a phenomenon of commercial apiaries resulting in problems for the beekeeper (Jay 1969a). However, this does not imply that drifting is insignificant under natural conditions. Colony aggregations of feral *Apis mellifera* honeybees have repeatedly been found (Oldroyd et al 1995). McNally & Schneider (1996) reported a considerable spatial aggregation of wild colonies of *Apis mellifera scutellata* (Lepeletier) in the Okavango River Delta (Botswana) which seems to result from limited opportunities for nesting sites. For the free nesting honeybee specie *Apis dorsata* nest aggregations of 2-120 colonies on a single tree or building are common (Seeley et al 1982, Ruttner 1988) and foreign workers have been shown using DNA microsatellites (Moritz et al 1995). Drifting has also been observed in the communal sweat bee *Agapostemon virescens* where 58% of adult females switch between nests (Abrams & Eickwort 1981) and in ants such as *Formica pratensis* (Neumann & Moritz, unpublished data). Hung & Rubik (1992) found up to 54.6% foreign workers in a feral population of Africanized *Apis mellifera* colonies.

Therefore, studies of the presence and origin of foreign individuals in honeybee colonies can provide basic understanding of the interactions between neighboring nests and subsequently for the evolution of colony integrity. Our estimators of drifting may be influenced by the various factors known for drifting in apiaries (Jay 1965, 1966a,b, 1968, 1969a, 1971 Currie & Jay 1988, Moritz & Neumann 1996 among others). However, we found no correlation between the amount of drift with the row position, showing that the effect of rows on drift (Jay 1966a, Currie & Jay 1988, Moritz & Neumann 1996) is reduced due to apiary layout. The non-detection error of drifted individuals in our sample was low as a result of to the high degree of heterozygosity at the used microsatellite loci. It might well be that the emigration and immigration of male sexuals are influenced by the size of mother and host colonies, because larger colonies produce more drones. But, there was no significant correlation between colony size and immigration of workers and drones in our sample which may result from sampling errors. Using DNA markers we may have misinterpreted a few worker produced drones as drifted individuals. However, the presence of successfully reproducing workers in queenright colonies is extremely rare due to worker policing (Ratnieks & Visscher 1989) but not impossible (Oldroyd et al 1994).

In the experiments of Currie & Jay (1988) the acceptance of artificially introduced drones in colonies ranged from 8 to 88% which is congruent with our findings of naturally immigrated drones. In our sample drones immigrated and emigrated significantly more often than workers. This supports Goetze (1954), Free (1958), Witherell (1965) and Currie (1982) who observed that drones drift more frequently than workers. This is also in line with findings that apiary layouts which significantly reduce the drifting of workers (Jay 1966a,b, 1968) do not necessarily reduce the proportion of drones that drift (Currie 1982). We could certainly not support reports that drones drift less often than workers (Butler 1939, Livenetz 1952, Lecômte 1958, Kepena 1963). The differences in the estimated amounts of drift reported in different studies could vary with the sampling technique used (Currie 1987) and the method of evaluating (Butler 1939, Ruttner 1992, Moritz & Neumann 1996), calculating or even defining "drift". The age of the drones at the time of the sampling (Currie 1987), the apiary layout used (Jay 1966b, 1968, Currie & Jay 1988), environmental conditions such as a nectar flow (Washington 1967), the topography of the study area (Currie 1982) and the race (Rinderer et al 1985, Ruttner 1992) may also be important. In all cases, only studies which simultaneously evaluate the drift of drones and workers, such as Free (1958) did, can provide comparable data. Free (1958) found that the average amount of drone drift is 2-3 times higher than that of workers. Our findings are in line with Free (1958) in principle, but we found 10 times more drifting drones which can be explained by the various factors influencing the amount of drift. Such high levels of foreign individuals in colonies made Accorti (1991) argue that honeybees have a marked tendency to "wander" among colonies. He proposed a hypothesis that there is a continuos interaction among the members of honeybee colonies present in a particular biotope.

The levels of adopted drone and worker individuals by the guard bees were independent from the level of polyandry of the queens. We found no significant correlation between the number of observed, estimated or effective queen matings and the level of immigrated and emigrated drones or workers. Thus, the degree of intracolonial genotypic diversity did not show any significant effect on the emigration and immigration of drones and workers in our tested colonies. Our result is in line with the findings of Beye *et al* (in press) for the meadow ant *Formica pratensis*. These authors did not find an influence of the intracolonial relatedness on the aggression behavior of workers. The common label acceptance model and the Gestalt model can both explain our findings. Beyond the number of queen matings which ensure a more or less unique colony odor under the Gestalt model it is not possible to distinguish between the two models. Moreover, the odor blend of a colony is highly complex. Random environmental effects such as the flower odor imported by the foragers (Getz 1991) certainly play a role. Breed (1983) has shown that both environmental and genetically factors are important. However, our results suggest that the foreign label rejection model is unlikely to explain our findings. We conclude that in *Apis mellifera* the intracolonial relatedness has no or only weak effects on the likelihood of foreign individuals to be adopted in host colonies. Instrumentally inseminated queens or queens which have naturally mated with an unusual low number of males (<10) as found by Neumann et al (in review) may provide a test system to further distinguish between the two models.

The impact of the amount of foreign bees on the tested colonial traits was weak and in no case significant. We found no significant correlation between the immigration of worker and drones and the honey yields of our colonies. So far, our results are in line with Matvijenko (1965), Jay (1969b) and Jay & Dixon (1988). Matvijenko (1965) found that orientation cues such as colors, which are known to reduce drift, did not effect the honey yields of colored and non colored colonies. Similarly, Jay (1969b) and Jay & Dixon (1988) found that apiary layouts which are known to reduce the drift of workers (Jay 1969a) did not affect the honey yield of the colonies in the studied apiaries. The effects of drifting found by Nekrasov (1949) and Robinson (1979) can be explained by: 1) a much higher amount of drifting workers up to 75 or 80% (Robinson 1979, Pfeiffer & Crailsheim 1994, Pfeiffer 1995), which is effected by the apiary layout (Jay 1966a) and 2) the positive correlation between colony size and honey yield known from routine beekeeping experience (Sachs 1964). In our study relevant worker population shifts between colonies are reduced due to apiary layout.

Given that the genotypic diversity of colonies has an impact on colony efficiency as predicted by the hypotheses for the evolution of polyandry (Crozier & Page 1985, Keller & Reeve 1994), one might expect a positive correlation between the immigration of workers and the honey yield of host colonies. Considering an average intracolonial relatedness between native honeybee workers of 0.28 (Neumann & Moritz, in review) and our findings of 0-14.29% foreign workers in colonies, which can be related by zero to the queen and her mates, the increase in genotypic variability due to foreign workers can be higher as resulting from polyandry alone (up to $r = 0.24$). Nevertheless, the increased intracolonial genotypic diversity did not significantly affect the colonial phenotype.

Surprisingly, we do not find any significant impact of drifting workers and drones on colony levels of infestation with *Varroa jacobsoni*. Although the intracolonial drone population can consist of more than 80% of foreign individuals no significant effect on colony levels of infestation with *V. jacobsoni* was found. So far, our results are in line with Goodwin et al (1994) who found that drifting is not a particularly cause of the spread of American foulbrood disease. But our results contrast to the findings of Sakofski (1991) who found higher emigration from colonies with higher levels of infestation with *V. jacobsoni*. However he used only small colonies (c. 1000 bees) which may interfere with the factors determining the amount of emigration. Honeybees invade colonies weakened by *V. jacobsoni* to rob their honey, which certainly promotes the propagation of that parasite. Indeed, robbing behavior seems to be the important spread mechanism for *V. jacobsoni*. Sakofski (1991) found when weak infested colonies were robbed by a strong uninfested colony, mites were transferred to the uninfested colony at a high transfer rate.

Given that the transmission of parasites and diseases via drifting is an important risk factor, honeybees should avoid the adoption of drifted individuals. They should carefully scrutinize incoming individuals because foreign individuals may carry the organisms that can potentially kill the colony. However, they do adopt new male nestmates very easily. We conclude that drifting of either drones and workers is not a particular cause for colony levels of infestation with *V. jacobsoni*. Such a high level of drifting drone honeybees is in line with the fact that honeybees do not show any particular behavioral pattern to avoid infestations with parasites and pathogens (Kraus & Page, in press).

Why do we find such a high amount of foreign drones in host colonies? Are drones simply more prone to orientation errors and/or are male sexuals more easily adopted by the guards of the host colony? Using DNA markers we can obviously not give a judgment about the behavioral interactions between the drifted individuals and the guards at the hive entrance. Furthermore, we do not know the actual number of individuals which are „lost“. Moritz and Neumann (1996) found that the underlying behavioral mechanisms for the orientation of workers are also important for drones. But, we can not exclude that drones are more prone to orientation errors. However, we have data about successfully adopted foreign bees. Thus, we can evaluate the effectiveness of the guards towards drones and workers by comparing the composition of the intracolonial drone and worker populations.

Since both drifting workers (Pfeiffer 1995) and drones (Kühl & Neumann 1996) significantly prefer their neighbouring colonies, we would expect the adoption rate to be equal to the rate of intruding individuals if the acceptance of the guards is less significant. Indeed, our colonies which show high levels of foreign drones also have a large proportion of male nestmates in other colonies. Thus, immigration and emigration of male sexuals were positive correlated. But this is not the case for workers. In light of this finding one could argue that the rejection mechanism found for workers is lacking for males altogether. However, this argument does not hold because the aggressive behavior of guard bees towards foreign workers and drones is similar (Kirchner and Gadagkar 1994). Furthermore, colonies which adopt a high number of workers do not necessarily adopt many foreign drones. Under the same adoption and/or rejection mechanism one would expect a common tendency for host colonies to accept foreign drones and workers. In particular we found a non significant negative In this study, we found colonies with more than 80% foreign drones. This may indicate that nestmate recognition is not particularly effective for drones. A potential guarding/rejection mechanism against males, if any, seems to be very inefficient because it failed at the average 50% of the time. On the other hand nestmate recognition is a common phenomenon in insect societies. For honeybees Breed *et al* (1982) and Breed (1983) showed that workers are able to discriminate nest mate workers from other workers. Also for males nestmate recognition has been documented in sweat bees, *Lasioglossum zephyrum* (Smith 1983) and in polistine wasps (Shellman-Reeve and Gamboa 1985).

This suggests that the adoption mechanism by the work force of host colonies is caste specific for workers and drones. Divergent olfactory cues may be important for the observed differences between immigration and emigration of workers and drones. Some authors claim a so-called "drone-pheromone" (Klinker 1993) which has been assumed to catalyze the adoption process for males. This indicates that the risk of being killed maybe low for a drifted male. This is supported by Currie (1987) who found that 20% of the drones drift more than once.

A simple explanation might be that drones were less often or less intensely screened by the guards. This seems plausible in light of the danger of robbing bees. Landing workers should be inspected immediately before they may enter the nest and could subsequently recruit new robbing bees. In this context, the different flight pattern of drifted and robbing bees may be different which can provide cues for the guards. Additionally, drones usually enter their mother colony after mating flights only for a very short period of time. They feed and leave the colony immediately for the next mating flight. Thus, intensive checks by the guards may potentially reduce the chance of a brother drone to mate. However, drifting workers entering a host colony were also accepted without antagonism in the experiments of Poltev (1968) and only few drifting workers were rejected or killed by the guard bees of recipient colonies as found by Jay & Dixon (1988). Pfeiffer & Crailsheim (1994) hypothesized from their observations that drifting did not shorten the lifetime of workers that drifted workers were rarely attacked by the guard bees. This suggests that the mechanism which enables the guards to distinguish between drifters and robbing bees is very efficient. Otherwise one would expect a higher amount of drifting workers being killed by the guards. But this may also suggest that there is no control/rejection mechanism at all for drifted workers and drones as soon as the guards have discriminated between robbing and drifted workers.

We hypothesize that simply the number of guard bees is the key factor for the amount of adopted drone and worker individuals. The more guard bees were present, the higher the chance of being checked. This seems to be supported by Echazaretta (1988, 1993) and Echazaretta et al (1989) who found a higher number of drones drifting from aggressive colonies to gentle ones and by the results of Eischen et al (1986) that attacks on intruding wax moths (*Galleria monella*) were correlated with the number of bees guarding the entrance. Echazaretta et al (1988) hypothesized that this reproductive parasitism would theoretically lead to a spread of defensive behavior in a population of honeybees. Indeed, European colonies have been found to have a higher number of immigrated drones than Africanized ones (Rinderer et al 1985) which show significantly more guard bees (Eischen et al 1986).

At first glance, the ready acceptance of foreign drones seems counterintuitive in light of inclusive fitness theory, particularly since the adoption of foreign drones is presumably not free of costs for the colony. However, behavioral patterns can only evolve if benefits outweigh the costs. None of the potential costs of hosting foreign individuals seem to be high enough to outweigh the costs of evolving an special effective guarding mechanism against drifting males and/or workers. No impact of drift on the level of the host colony's phenotype has been found and as long as there are no distinct differences in the aggression/acceptance level of host colonies for drones a male reproductive parasitism (Rinderer et al 1985) is not fitness relevant.

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References

Abrams J, Eickwort GC (1981) Nest switching and guarding by the communal sweat bee *Agapostemon virescens* (Hymenoptera: Halictidae). Insectes Socieaux 28 (2): 105-116

Accorti M (1991) Indagine preliminare sulla deriva delle api. Apicoltura 7, 1-15

Beye, M; Raeder, U (1993) Rapid DNA preparation from bees and % GC fractionation. Bio Techniques 14 (3), 372-374

Beye M, Neumann P, Chapuisat M, Pamilo P, Moritz RFA (1998): Nestmate recognition and genetic relatedness in the ant *Formica pratensis*. Behav Ecol Sociobiol, in press

Bayerische Landesanstalt für Bienenzucht (1995) Leistungsprüfung 1993-1995, Prüfhof Schwarzenau, Abschlußbericht. Bayerische Landesanstalt für Bienenzucht, Erlangen, Germany

Boylan-Pett W, Ramsdell DC, Hoopinggarner RA, Hancock JF (1991) Honeybee foraging behaviour, in-hive survival of infectious, pollen-borne blueberry leaf mottle virus and transmission of the virus in highbush blueberry. Pythopathology 81 (11): 1407-1412

Brandenburgo MAM, Gonçalves LS (1990) Environmental influence on the aggressive (defence) behaviour and colony development of Africanized bees (*Apis mellifera*). Ciência e Cultura 42 (10): 759-771

Breed MD (1983) Nestmate recognition in honeybees. Anim. Behav. 31: 86-91

Breed MD, Julian GE (1992) Do simple rules apply in honey-bee nestmate recognition? Nature 357: 685-686

Breed MD, Smith TA, Torres A (1992) Role of guard honeybees *Apis mellifera* L. (Hymenoptera: Apidae) in nestmate discrimination and replacement of removed guards. Ann Entomol Soc Am 85: 633-647

Buren NWM, Mariën AGH, Oudejans RCHM, Velthuis HHW (1992) Perizin an acaricide to combat the mite *Varroa jacobsoni*: its distribution in and influence on the honeybee *Apis mellifera*. Physiological Entomology 17 (3): 288-296

Butler C.G. (1939) The drifting of drones. Bee World 20:140-142

Butler C.G., Free JB (1952) The behaviour of worker honeybees at the hive entrance. Behaviour 4, 262-292

Cornuet JM, Aries F (1980) Number of sex alleles in a sample of honeybee colonies. Apidologie 11: 87-93

Crozier RH, Dix MW (1979) Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. Theor Popul Biol 10: 1-9

Crozier RH, Page RE Jr. (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. Behav Ecol Sociobiol 18: 105-115

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford UK

Currie RW (1982) Some factors affecting the orientation of drone honeybees. MSc thesis, University of Manitoba, Canada

Currie RW (1986) The effects of the position and apparent movement of the sun and a colony's queen state on the orientation of drone honey bees (*Apis mellifera* L.) to their hives. Ph.D. Thesis, University of Manitoba, Canada

Currie RW (1987) The biology and behaviour of drones. Bee world 68, 129-143

Currie RW and Jay SC (1988) The influence of a colony's queen state, time of the year, and drifting behaviour, on the acceptance and longevity of adult drone honeybees (*Apis mellifera* L.). Journal of Apicultural Research 27 (4): 219-226

Currie RW and Jay SC (1991) The influence of a colony's queen state on the drifting of drone honey bees (*Apis mellifera* L). Apidol 22: 183-195

Currie RW and Jay SC (1991) Drifting behaviour of drone honey bees (*Apis mellifera* L). in commercial apiaries. Journal of Apicultural Research 30 (2): 61-68

Duranville C, Miniggio C, Arnold G, Borneck R (1991) Dérive des butineuses et réinfestation des colonies d'abeilles par *Varroa jacobsoni*. Santé de l'Abeille 125, 228-235

Echazaretta CM (1988) The relationship between drone drifting and aggression in honeybee colonies (*Apis mellifera* L.). MSc Thesis, University of Wales College of Cardiff, UK

Echazaretta CM, Paxton RJ, Kolmes SA, Ferguesson-Kolmes LA (1989) Colony defense and male behaviour in honeybees *Apis mellifera*. In Actes des Colloques Insectes Sociaux, Vol. 5. Compte rendu Colloque annuel. Londres, 20-22 Sept. 1988. British Section IUSSI, Section Française UIEIS. 153-159. ISBN 2-905272-02-5

Echazaretta CM (1993) The biology and reproductive behaviour of Africanized and European honey bee drones (*Apis mellifera* L.) in tropical Yucatan Mexico. Ph.D. thesis, University of Wales College of Cardiff

Estoup A., Solignac M., Harry M., Cornet J.M. (1993): Characterisation of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*, NAR, 21, 6, 1427-1431

Estoup, A; Solignac; M; Cornuet; J.M (1994) Precise assessment of the number of patrilines and genetic relatedness in honeybee colonies. Proc R Soc L B 258: 1-7

Free JB (1958) The drifting of honeybees. J Agric Sci 51: 294-306

Free JB, Spencer Booth Y (1961) Further experiments on the drifting of honeybees. J Agric Sci 57:153.

Giray-T; Robinson-G-E (1996) Common endocrine and genetic mechanisms of behavioral development in male and worker honey bees and the evolution of division of labor. Proceedings of the National Academy of Sciences of the United States of America 93(21): 11718-11722

Getz WM (1991) The honeybee as a model kin recognition system. In: Kin recognition. Cambridge University Press, Cambridge, UK, p 358-412

Goetze G. (1954) How much do drones drift in large apiary? Leipziger Bienenzeitung (West) 68, 9, 223-225 (Abstract in Bee World 36 (6), 115)

Goodwin RM, Perry JH and Brown P (1993) American foulbrood disease. Part 3: spread. New Zealand Beekeeper 219: 7-10

Goodwin RM, Perry JH and Houten AT (1994) The effect of drifting honey bees on the spread of American foulbrood infections. J Apic Res 33(4): 209-212

Greenberg L (1979) Genetic component of bee odor in kin recognition. Science 206: 1095-7

Hamilton WD (1964a) The genetical evolution of social behaviour I. J. Theor. Biol. 7:1-16

Hamilton WD (1964b) The genetical evolution of social behaviour II. J. Theor. Biol. 7:17-52

Henderson CE (1994) Influence of the presence of adult drones on the further production of drones in honey bee (*Apis mellifera* L) colonies. Apidol 25: 31-37

Hepburn R, Allsopp M (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*: South African J. Sci. 90: 247-249

Hung ACF, Rubik WL (1992) Biochemical evidence of non-progeny workers in feral Africanized honey bee (*Apis mellifera* L.) colonies (Hymenoptera: Apidae). Bee Science 2 (1): 33-36

Jay SC (1965) Drifting of honeybees in commercial apiaries. I. Effect of various environmental factors. J. Apic. Res. 4:167-175

Jay SC (1966a) Drifting of honeybees in commercial apiaries. II. Effect of various factors when hives are arranged in rows. J. Apic. Res. 5:103-112

Jay SC (1966b) Drifting of honeybees in commercial apiaries. III. Effect of apiary layout. J. Apic. Res. 5:137-148

Jay SC (1968) Drifting of honeybees in commercial apiaries. IV. Further studies on the effect of apiary layout. J. Apic. Res. 7:37-44

Jay SC (1969a) The problem of drifting in commercial apiaries. Am Bee J 109: 178-179114

Jay SC (1969b) Drifting of honeybees in commercial apiaries. V. Effect of drifting on honey production. J. Apic. Res. 8: 13-17

Jay S.C. (1971) Sun position as a possible factor in the disorientation of honeybees (*Apis mellifera*) (Hymenoptera: Apidae). Can Entomol 103: 1111-1114

Jay SC, Dixon D. (1988) Drifting behaviour and honey production of honeybee colonies maintained on pallets. J. Apic. Res. 27(4):213-218

Kepena L. (1963) Beitrag zur Biologie der Drohnen-Bienen des Stammes "Tatranka", 19 Int. Bz. Kongr. Prag 1963, 241-242

Kirchner W.H., Gadagkar R. (1994) Discrimination of nestmate workers and drones in honeybees. Insectes Socieaux 41: 335-338

Klinker K (1993) Drohnenakzeptanz in Völkern von *Apis mellifera* L.. MSc thesis, JW Goethe-Universität, Frankfurt/Main, Germany

Kraus B, Page RE Jr. (in press) Parasites, pathogens and polyandry in social insects. Am Nat

Kryger P (1997) The impact of intracolonial genetic relationship on the state of the honeybee family. Ph.D. thesis, Technische Universität Berlin, Germany

Kurrenoi MN (1956) [flight activity and sexual maturity of drones], Pchelovodsto 12, 24-28 in Russian. Abstract in Bee world 37 (7): 160

Kühl A, Neumann P (1996) Analysis of the drifting of drones at an apiary using single locus DNA-fingerprinting. Apidol 27 (4): 301-302

Lacy RC, Sherman PW (1983) Kin recognition by phenotype matching. Am Nat 121: 489-512

Lecômte J. (1958) Que savons-nous du comportement des faux bourdons?, Ann. Abeille 1, 1, 31-39

Livenetz, T.P. (1952) [The drifting of drones] Pchelowodstwo 29:25-30 in Russian. Abstract in Bee World 33:142

Matheson, A (1984) Practical beekeeping in New Zealand. Government Printing Office, Wellington New Zealand; 185 pp.

Matvijenko A (1965) [Effect of coloured orientation surfaces on drifting, and on honey yield] ČSAZV 4: 105-112 in Czech; English summary

McNally LC, Schneider SS (1996) Spatial distribution and nesting biology of colonies of the African honey bee *Apis mellifera scutellata* (Hymenoptera: Apidae) in Botswana, Africa. Environmental Entomology 25(3): 643-652

Moritz RFA, 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

Moritz RFA and Neumann P (1996) Genetic analysis of the drifting of drones in *Apis mellifera* using multilocus DNA fingerprinting. Ethology 102, 580-590

Moritz RFA, Kryger P, Allsopp MH (1996) Competition for royalty in bees. Nature 384: 31

Mossadegh MS (1993) [The biology of the tracheal mite *Acarapis woodi* (Acari: Tarsonemidae) and role of drone honey bees in intercolony spread of the mite, in North Carolina.] Scientific Journal of Agriculture 16 (1/2) 2-3: 14-22 in Arab with English Abstract..

Neumann P, van Praagh JP, Moritz RFA, Dustmann J (in review) Testing the reliability of the potential honeybee mating area Baltrum using virgin queens and single-locus DNA-fingerprinting Submitted to *Apidologie*

Neumann P, Moritz RFA, van Praagh J (in review) Queen mating-frequency on different types of honeybee mating yards. Submitted to J Apic Res

Neumann P, Kryger P, Moritz RFA (in review) Variability of queen mating-frequency in *Apis mellifera.* Submitted to Mol Ecol

Oertel E (1956) Observations on the flight of drone honey bees, Ann. Entomol. Soc. Am. 49, 497-500

Oldroyd BP, Smolenski AJ, Cornuet JM & Crozier RH (1994) Anarchy in the beehive. Nature 371: 749

Oldroyd BP, Smolenski A, Lawler S, Estoup A, Crozier R (1995) Colony aggregations in *Apis mellifera*. Apidol 26, 119-130

Oldroyd PB, Clifton MJ, Wongsiri S, Rinderer TE, Sylvester HA, Crozier RH (1997) Polyandry in the genus *Apis*, particularly *Apis andreniformis*. Behav Ecol Sociobiol 40: 17-26

Pfeiffer K, Crailsheim K (1994) Survival of honeybees after drifting into related or nonrelated colonies. In: Les Insectes Socieaux (Lenoir A, Arnold G & Lepage M eds) Univ Paris Nord, Villetaneuse, 498

Pfeiffer K (1995) Das Verfliegen und Überleben von Honigbienen (*Apis mellifera*) im Hinblick auf ihr Verwandtschaftsverhältnis. MSc. thesis. Karl-Franzens-Universität Graz

Poltev VL (1968) [Robbing and drifting of honeybees], Pchelevodsto 88 (7): 42 in Russian. Abstract in Apicultural Abstracts 1973, 24 (2): 78

Ratnieks FLW, Visscher PK (1989) Worker policing in the honeybee. Nature 342: 796-797

Rath W, Delfinado-Baker M, Drescher W (1991) Observations on the mating behavior, sex ratio, phoresy and dispersal of *Tropilaelaps clareae* (Acari: Laelapidae). International Journal of Acarology 17 (3): 201-208

Ratnieks FLW, Visscher P K (1989) Worker policing in the honey bee. Nature 342: 796-797

Rauschmayer F (1928) Das Verfliegen der Bienen und die optische Orientierung am Bienenstand, Arch. Bienenk. 9, 249-322

Robinson FA (1979) Foraging efficiency and drift among honey bee colonies. In: Proceedings of the Fourth International Symposium on Pollination, Maryland 1978

Rinderer T.E., Hellmich R.L., Danka R.G., Collins A.M. (1985) male reproductive parasitism: A factor in the Africanization of European honey bee populations, Science 228, 1119-1121

Ruttner F (1966) The life and flight activity of drones, Bee world 47, 3, 93-100

Ruttner F (1988) Biogeography and taxonomy of honeybees. Springer, Heidelberg New York Berlin

Ruttner F (1992) Naturgeschichte der Honigbienen. Ehrenwirth, München

Sachs HG (1964) Vergleich der Honigerträge von Bienenvölkern der Carnica-Rasse bei unterschiedlicher Raumumgebung und unterschiedlicher Volksstärke. Z Bienenforsch 7 (2): 47-52

Sakofski F (1991) Quantitative investigations on transfer of *Varroa jacobsoni* Oud. In: Proceedings of the International Symposium on Recent Research on Bee Pathology, September 5-7, 1990, Ghent, Belgium [edited by Ritter WJ]. Merelbeke, Belgium; Rijksstation voor Nematologie en Entomologie on behalf of Apimondia 70-72

Schmidt J (1995) Der Einfluß einer verkürzten Brutphase bei *Apis mellifera* LINNÉ auf die Überwinterung und Frühjahrsentwicklung der Bienenvölker und auf die Populationsentwicklung von *Varroa jacobsoni* OUDEMANS. MSc thesis, JW Goethe-Universität, Frankfurt/Main, Germany

Seeley TD, Seeley RH, Aratanakul P (1982) Colony defense strategies of the honeybees in Thailand. Ecol Monogr 52: 43-63

Shellman-Reeve J, Gamboa GJ (1985) Male social wasps (*Polistes fuscatus*, Hymenoptera: Vespidae) recognize their male nest mates. Anim Behav 33, 331-333

Smith BH (1983) Recognition of female kin by male bees through olfactory signals. Proc Nat Acad Sci USA 80: 4551-4553

Southwick EE, Moritz RFA (1985) Metabolic response to alarm pheromone in honeybees. J Insect Physiol 31: 389-392

Thyri H (1973) [Drifting in the apiary], Tidskrift for Biavl 107 (1): 14-16 in Norwegian. Abstract in Apicultural Abstracts 1974 25 (4): 185

Stuart RJ (1987) Transient nestmate recognition cues contribute to a multicolonial population structure in the ant, *Leptothorax curvispinosus*. Behav Ecol Sociobiol 21, 229-235

Stuart RJ (1988) Development and evolution in the nestmate recognition systems of social insects. In: Evolution of Social Behaviour and Integrative Levels (Ed. By G. Greenberg & E. Tobach), pp. 177-195. Hillsdale, New Jersey: Erlbaum.

Stuart RJ (1993) Differences in aggression among sympatric, facultatively polygynous *Leptothorax* ant species. Anim Behav 46, 809-812

Vollbehr J (1975) Zur Orientierung junger Honigbienen bei ihrem ersten Orientierungsflug. Zool. Jb. Physiol. 79:33-69

Waldmann B, Frumhoff PC, Sherman PW (1988) Problems of kin recognition. Trends Ecol Evol 3: 8-13

Washington RJ (1967) Drones, American Bee Journal 108 (9), 7-9

Witherell PC (1965) Drifting of drones. Amer Bee J 105:175

Table 1 Putative genotypes (four microsatellite loci, length in base pairs) of the 38 tested queens (Neumann et al, in review, in review, Neumann & Moritz in review).

Locus Colony	A76	A107	B124	A43	Locus Colony	A76	A107	B124	A43
1	209	172	216	127	20	277	170	214	127
	281	174	216	140		291	176	214	146
2	251	158	214	127	21	267	170	214	140
	313	164	218	127		283	176	216	140
3	257	165	214	127	22	239	160	214	127
	267	171	214	140		283	176	216	140
4	251	171	214	127	23	251	160	214	127
	261	177	214	140		265	168	222	140
5	249	171	220	127	24	243	158	214	126
	313	176	230	140		251	160	216	140
6	229	176	214	127	25	209	162	214	127
	277	183	214	146		271	162	216	140
7	253	162	214	127	26	209	172	214	127
	267	174	214	140		283	174	216	140
8	251	160	214	127	27	251	160	214	127
	305	160	222	140		259	172	218	140
9	251	168	216	126	28	305	166	214	140
	313	172	218	140		317	176	216	142
10	209	160	214	127	29	287	159	214	127
	271	168	224	140		313	176	216	140
11	259	170	214	127	30	285	159	214	140
	283	176	216	140		299	183	214	146
12	277	158	214	127	31	287	166	214	127
	283	176	216	140		343	168	214	140
13	239	158	214	127	32	267	160	214	127
	299	162	216	140		267	170	222	140
14	209	162	214	140	33	271	165	214	127
	343	172	222	140		313	167	214	140
15	209	162	212	126	34	209	170	214	127
	283	172	216	140		277	173	216	140
16	253	167	214	140	35	299	168	214	127
	295	171	216	140		313	174	216	140
17	299	167	218	127	36	251	158	214	127
	299	171	222	140		287	163	214	140
18	261	165	218	140	37	251	158	214	127
	271	177	222	140		287	163	218	140
19	265	163	214	127	38	267	160	212	127
	287	165	216	140		343	160	214	140

Table 2 Genotypes (four microsatellite loci, length in base pairs) of the drifted workers, their host colonies and their putative mother colonies (nd = not determined, ? = no corresponding queen genotype).

Locus Worker	A76	A107	B124	A43	Host Colony	Mother Colony
1	299	168	214	127	1	36
	299	176	216	142		
2	271	162	214	140	1	29
	313	176	216	140		
3	313	168	216	127	2	35
	313	172	216	140		
4	271	168	214	140	3	10
	271	168	214	140		
5	249	176	nd	nd	4	5
	313	176	nd	nd		
6	229	168	214	140	4	6
	259	183	214	146		
7	271	168	214	127	4	35
	313	174	220	140		
8	229	162	214	127	4	7
	253	168	214	140		
9	233	171	214	127	4	3
	267	176	218	139		
10	275	158	214	127	5	12
	283	172	214	127		
11	271	171	222	127	5	17
	299	171	230	127		
12	271	171	212	127	5	?
	343	171	214	140		
13	249	172	214	127	5	26
	283	172	216	140		
14	271	158	214	126	5	12
	283	179	216	127		
15	283	176	216	127	5	12
	313	176	218	140		
16	271	171	214	127	6	18
	271	177	222	140		
17	271	166	214	127	6	34
	277	173	214	140		
18	249	158	220	127	6	5
	271	176	220	140		
19	251	158	214	127	7	36
	287	164	214	127		
20	249	158	214	nd	7	18
	271	176	218	nd		
21	229	170	214	127	7	6
	273	176	218	139		
22	267	168	214	126	7	?
	283	174	214	146		
23	249	160	214	140	9	14
	343	172	216	140		
24	269	168	214	140	13	31
	343	174	214	140		
25	251	162	214	140	13	24
	251	183	214	140		
26	271	165	214	140	14	33
	271	172	222	140		
27	239	160	214	140	15	22
	305	170	214	140		
28	267	158	214	140	16	36
	287	167	214	140		
29	287	163	214	127	17	19
	305	176	218	127		
30	287	165	214	127	17	19
	287	165	218	140		
31	267	163	214	127	17	19
	305	176	222	140		
32	267	171	214	127	17	3
	299	171	216	140		
33	287	161	214	140	18	19
	313	165	218	140		
34	271	159	214	127	18	29
	299	169	228	146		
35	271	160	214	127	18	10
	287	177	216	140		
36	271	165	214	127	19	33
	299	171	214	140		
37	267	170	218	nd	20	32
	267	179	222	nd		
38	229	160	214	146	22	30
	299	167	216	146		
39	295	167	214	127	22	16
	303	182	214	140		
40	267	164	nd	nd	23	21
	271	176	nd	nd		
41	243	160	214	127	23	24
	305	160	214	140		
42	277	168	nd	127	26	34
	277	172	nd	127		
43	251	162	214	126	26	25
	271	172	216	127		
44	277	170	214	140	27	34
	325	176	216	140		
45	249	164	214	140	27	23
	267	168	214	140		
46	241	nd	214	140	27	24
	243	nd	214	140		
47	271	165	214	nd	29	33
	277	177	214	nd		
48	251	160	nd	nd	29	23
	271	183	nd	nd		
49	243	158	nd	nd	29	24
	277	163	nd	nd		
50	251	160	nd	nd	29	27
	277	172	nd	nd		
51	239	168	214	127	30	22
	267	176	214	140		
52	229	176	214	127	30	29
	287	176	216	140		
53	236	162	214	127	30	29
	313	176	214	140		
54	257	160	214	127	30	27
	277	172	216	140		
55	261	158	212	127	31	?
	261	168	214	140		
56	265	163	216	127	33	19
	265	163	216	127		
57	313	164	214	127	33	2
	343	167	222	140		
58	209	168	nd	nd	33	10
	209	168	nd	nd		
60	277	174	216	127	34	35
	311	174	224	127		
61	265	174	216	127	34	35
	311	174	216	127		
62	265	174	216	127	34	35
	299	176	216	140		
63	251	163	214	127	35	36
	283	167	216	140		
64	305	171	214	127	35	?
	339	177	216	127		
65	265	160	214	127	35	38
	267	166	214	140		
66	287	158	214	127	36	37
	311	171	216	140		
67	271	168	214	127	36	35
	299	174	214	140		
68	271	168	214	127	37	35
	311	174	214	140		
69	267	160	214	127	38	32
	267	174	214	140		
70	243	160	214	140	38	24
	265	176	214	140d		

Table 3 Genotypes (four microsatellite loci, length in base pairs) of the drifted drones, their host colonies and their putative mother colonies. If more than one queen genotype is corresponding, the possibilities are given (nd = not determined, ? = no corresponding queen genotype).

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Locus Drone	A76	A107	B124	A43	Host Colony	Mother Colony	Locus Drone	A76	A107	B124	A43	Host Colony	Mother Colony
1	nd	nd	230	127	3	5	128	nd	nd	222	140	19	8,17,32
2	269	177	214	127	3	?	129	261	165	222	140	20	18
3	275	177	214	140	3	12	130	261	177	218	140	20	18
4	313	171	nd	nd	3	5	131	261	177	218	140	20	18
5	313	176	220	140	4	5	132	271	177	218	140	20	18
6	313	176	nd	nd	4	5,29	133	261	177	222	140	20	18
7	313	176	nd	nd	4	5,29	134	271	165	222	140	20	18
8	253	162	214	127	4	7	135	261	177	218	140	20	18
9	257	165	nd	nd	4	3	136	261	166	218	140	20	18
10	253	162	nd	nd	4	7	137	271	166	222	140	20	18
11	313	176	220	140	4	5	138	271	166	222	140	20	18
12	229	176	nd	nd	4	6	139	265	163	216	127	20	19
13	245	170	nd	nd	4	5	140	293	168	218	127	20	17
14	229	176	nd	nd	4	6	141	271	176	214	146	20	?
15	nd	nd	220	140	4	5,27	142	271	177	218	140	20	18
16	275	176	nd	nd	4	6,20	143	271	165	218	140	20	18
17	267	171	216	140	5	3	144	271	165	222	140	20	18
18	251	174	214	140	6	7	145	271	177	218	140	20	18
19	267	171	214	127	6	3	146	271	177	222	140	20	18
20	311	170	230	127	6	5	147	261	177	218	140	20	18
21	249	176	220	140	6	5	148	299	170	214	127	20	35
22	313	171	220	140	6	5	149	265	163	214	127	20	19
23	249	171	230	140	6	5	150	265	165	214	127	20	19
24	251	171	214	127	7	4	151	287	165	216	127	20	19
25	251	177	nd	nd	7	4	152	265	165	216	127	20	19
26	229	183	214	146	7	6	153	271	165	218	140	20	18
27	313	176	230	127	7	5	154	287	165	216	127	20	19
28	275	183	214	127	7	6	155	229	176	214	127	20	6
29	265	165	nd	nd	17	19	156	287	163	216	127	20	19
30	265	165	nd	nd	17	19	157	271	177	218	140	20	18
31	265	163	nd	nd	17	19	158	261	165	nd	nd	20	18
32	275	176	214	127	17	6,20	159	313	168	216	127	34	35
33	275	176	214	146	17	6	160	313	168	216	127	34	35
34	271	165	nd	nd	17	33	161	271	165	nd	nd	34	33
35	271	165	nd	nd	17	33	162	299	168	nd	nd	34	35
36	275	176	nd	nd	17	12,20	163	251	163	nd	nd	34	36,37
37	271	177	218	140	17	18	164	271	165	nd	nd	34	33
38	265	163	nd	nd	17	19	165	251	163	nd	nd	34	36,37
39	271	165	218	140	17	18	166	313	174	216	127	34	35
40	291	176	214	146	17	20	167	313	168	214	140	34	35
41	265	165	216	140	17	19	168	313	168	216	127	34	35
42	209	170	nd	nd	17	34	169	313	168	216	140	34	35
43	291	176	214	146	17	20	170	313	174	216	140	34	35
44	275	176	nd	nd	17	12,20	171	313	174	216	140	34	35
45	291	176	214	127	17	20	172	299	174	214	127	34	35
46	291	176	214	146	17	20	173	313	174	216	127	34	35
47	291	176	214	140	17	20	174	251	163	218	140	34	37
48	261	171	nd	nd	17	4	175	313	168	nd	nd	34	35
49	271	165	nd	nd	17	18	176	251	163	218	127	34	37
50	nd	nd	214	140	17	3,7,16,..	177	285	158	214	140	34	36
51	251	171	nd	nd	17	4	178	343	160	214	140	34	38
52	291	170	nd	nd	17	20	179	299	168	216	127	34	35
53	265	163	nd	nd	17	19	180	335	162	216	140	34	?
54	287	163	214	140	17	19	181	283	160	214	140	34	22
55	305	167	214	127	17	?	182	313	174	216	127	34	35
56	265	163	216	140	17	19	183	287	163	214	127	34	19,37
57	261	165	nd	nd	17	18	183	295	176	224	127	34	?
58	nd	nd	214	140	17	3,7,16,..	185	313	174	214	127	34	35
59	299	176	nd	nd	17	35	186	209	172	216	140	34	1
60	275	176	214	146	17	6	187	271	163	214	140	35	33
61	287	165	214	127	17	19	188	285	160	214	127	35	36
62	265	166	216	127	18	19	189	287	163	214	140	35	19,37
63	305	171	216	140	18	?	190	271	162	214	140	35	25
64	295	167	216	140	18	16	191	277	173	216	127	35	34
65	265	163	216	127	18	19	192	277	173	216	140	35	34
66	287	165	214	127	18	19	193	299	167	218	127	35	17
67	305	166	216	140	18	28	194	277	173	216	127	35	34
68	265	168	214	127	18	23	195	343	160	212	140	36	38
69	nd	nd	216	127	18	1,3,19,...	196	277	170	216	127	36	34

70	287	169	214	127	18	31	197	271	161	214	140	36	10
71	287	163	216	127	18	19	198	nd	nd	212	140	36	38
72	265	163	216	127	18	19	199	343	160	214	127	36	38
73	291	176	214	146	18	20	200	287	163	216	127	36	19
74	287	165	214	127	18	19	201	277	170	214	127	36	34
75	287	165	216	127	18	19	202	nd	nd	218	140	36	9,17,...
76	261	178	222	140	18	?	203	299	174	214	140	36	35
78	265	165	214	127	18	19	204	343	161	214	140	36	38
79	305	171	216	140	18	?	205	299	168	216	140	36	35
80	291	176	214	127	18	20	206	277	173	212	140	36	?
81	nd	nd	214	127	18	2,3,19,..	207	343	160	214	127	36	38
82	287	164	216	127	18	19	208	343	160	212	140	36	38
83	287	165	214	127	18	19,31	209	209	173	216	140	37	34
84	265	165	214	127	18	19	210	343	162	214	140	37	14
85	265	163	216	127	18	19	211	287	159	216	127	37	29
86	265	165	214	127	18	19	212	299	168	216	127	37	35
87	nd	nd	216	140	18	1,11,16.	213	271	162	214	140	37	25
88	nd	nd	214	127	18	2,3,19,...	214	271	162	212	140	37	?
89	271	165	222	140	19	18	215	nd	nd	212	140	37	38
90	261	165	218	140	19	18	216	343	160	214	127	37	38
91	271	165	222	140	19	18	217	251	158	214	140	37	36
92	261	165	218	140	19	18	218	277	173	216	127	37	34
93	271	177	222	140	19	18	219	313	176	214	140	37	29
94	291	170	214	127	19	20	220	313	176	220	140	37	5
95	271	177	222	140	19	18	221	287	168	218	127	37	?
96	291	176	214	127	19	20	222	251	158	214	127	37	36
97	273	170	214	146	19	20	223	271	162	214	140	37	25
98	261	165	218	140	19	18	224	299	169	216	127	37	35
99	271	177	218	140	19	18	225	271	162	212	140	37	?
100	271	165	218	140	19	18	226	285	158	214	140	37	36
101	271	177	222	140	19	18	229	271	162	212	140	37	?
102	271	165	222	140	19	18	228	271	162	214	140	37	25
103	271	177	222	140	19	18	229	299	174	216	127	37	35
104	271	177	222	140	19	18	230	285	158	214	140	37	36
105	299	168	216	140	19	35	231	285	159	216	127	37	29
106	299	167	218	140	19	17	232	271	162	214	140	37	25
107	271	177	222	140	19	18	233	287	158	214	140	37	36
108	271	177	218	140	19	18	234	299	174	214	140	37	35
109	291	170	214	146	19	20	235	251	163	214	140	38	36,37
110	291	170	214	127	19	20	236	251	158	214	140	38	36,37
111	271	177	218	140	19	18	237	277	173	216	140	38	34
112	291	176	214	146	19	20	238	311	176	214	127	38	29
113	nd	nd	218	140	19	9,17,37	239	313	168	nd	nd	38	9,35
114	261	177	222	140	19	18	240	251	163	214	140	38	36,37
115	271	165	218	140	19	18	241	251	164	212	140	38	?
116	271	165	222	140	19	18	242	287	160	212	140	38	?
117	261	165	218	140	19	18	243	nd	nd	214	127	38	2,3,19,..
118	271	177	nd	nd	19	18	244	251	160	218	140	38	27
119	271	177	218	140	19	18	245	343	162	216	127	38	?
120	261	177	218	140	19	18	246	209	173	214	140	38	34
121	271	177	222	140	19	18	249	313	168	216	127	38	35
123	291	176	214	146	19	20	248	299	174	214	140	38	35
124	271	165	218	140	19	18	249	287	163	214	140	38	37
125	nd	nd	222	140	19	8,17,32	250	271	162	216	127	38	25
126	nd	nd	218	140	19	9,17,37,	251	209	172	nd	nd	38	1,14
127	nd	nd	222	140	19	8,17,32	252	287	163	nd	nd	38	19,37

Table 4 Emigration and immigration of workers and drones for the tested colonies (Emigration = E, Immigration = I, N = total sample size per colony).

	Drones			Workers		
Colony	E	I	N	E	I	N
1				0	10	30
2				3,03	0	33
3	18,18	26,67	14	5	3,23	39
4	36,36	63,16	20	0	8,8	61
5	22,45	2,56	39	5,56	14,29	40
6	28,57	28,57	20	5,41	7,5	38
7	27,27	38,46	13	1,69	6,45	62
8				0	0	22
9				0	3,13	32
10				8,57	0	32
11				0	0	32
12				2,56	2,63	38
13	77,42	68,89	43	2,7	10	37
14	77,42	65	35	5,41	7,5	40
15	85,29	89,13	42	11,36	2,5	40
16	53,85	71,43	42	0	2,56	29
17				9,68	0	34
18				0	7,41	27
19				4	3,85	23
20				0	3,85	33
21				3,03	0	24
22				7,69	6,9	31
23				6,45	0	34
24				13,51	0	32
25				3,03	0	26
26				3,7	6,67	29
27				6,9	10,71	35
28				0	0	30
29				11,76	12,12	30
30				3,7	13,33	46
31				2,56	2,56	43
32				4,55	0	36
33				6,67	6,67	42
34	34,48	59,57	42	7,69	7,14	41
35	49,06	22,86	33	15,22	7,5	41
36	17,07	29,17	45	9,5	5	40
37	33,33	81,25	30	2,5	2,5	40
38	39,13	56,25	28	2,56	2,38	37
x/Σ	42,85	50,21	449	4,63	4,66	1359
± s.e,	6,07	6,8		0,66	0,69	

Table 5 Allele frequencies for the tested honeybee population. Only identified sexual reproductives are considered.

Locus A76			**Locus A107**		
Allele (bp)	Queens (n = 76)	Queens and drones (n = 743)	Allele (bp)	Queens (n = 76)	Queens and drones (n = 753)
209	0.09	0.016	141	0	0.007
231	0.01	0.04	158	0.079	0.085
233	0	0.013	159	0.026	0.025
239	0.03	0.015	160	0.132	0.114
243	0.01	0.067	162	0.079	0.066
249	0.01	0.018	163	0.039	0.033
251	0.12	0.073	164	0.013	0.056
255	0.03	0.021	165	0.053	0.02
259	0.04	0.05	166	0.026	0.066
261	0.03	0.044	167	0.039	0.039
265	0.03	0.077	168	0.066	0.076
267	0.08	0.051	169	0	0.009
271	0.05	0.081	170	0.066	0.068
277	0.04	0.028	171	0.066	0.045
279	0	0.011	172	0.066	0.064
281	0.01	0.011	173	0.013	0.015
283	0.08	0.047	174	0.053	0.021
287	0.08	0.047	175	0	0.042
289	0	0.003	176	0.132	0.076
291	0.01	0.015	177	0.026	0.053
295	0.01	0.022	181	0	0.012
299	0.08	0.059	183	0.026	0.007
305	0.03	0.031	189	0	0.003
311	0	0.02			
313	0.09	0.073			
325	0	0.007			
331	0	0.004			
343	0.04	0.037			
353	0	0.026			

Locus B 124			**Locus A43**		
Allele (bp)	Queens (n = 76)	Queens and drones (n = 739)	Allele (bp)	Queens (n = 76)	Queens and drones (n = 715)
212	0.03	0.022	124	0	0.001
214	0.54	0.507	126	0.039	0.098
216	0.24	0.242	127	0.395	0.386
218	0.08	0.1	139	0	0.015
220	0.01	0.056	140	0.513	0.443
222	0.08	0.031	141	0	0.007
224	0.01	0.009	142	0.013	0.01
228	0	0.008	146	0.039	0.039
230	0.01	0.016			
232	0	0.001			
234	0	0.007			

Table 6 Correlation matrix (simple correlation, r-matrix) for the immigration and emigration of workers and drones with the row position (as defined by Moritz & Neumann 1996), with the number of observed (n_o), estimated (*k*) and effective (n_e) queen matings (data from Neumann *et al*, in review, in review, Neumann & Moritz, in review, Neumann & Moritz previously unpublished) and colony size (Size 94, Size 95). Partial correlations (corrected for colony size 1994 and 1995) were performed between the drift and the performance data of the tested honeybee colonies (Bayerische Landesanstalt für Bienenzucht 1995; Neumann & Moritz, in review). Colony levels of infestation with *Varroa jacobsoni* (*Varroa* 94, *Varroa* 1995) and the honey yield of colonies (Honey 94, Honey 95) were considered. No significant correlations were found.

	Emigration		Immigration	
	Drones	Workers	Drones	Workers
Row position	-0.13	-0.25	0.22	-0.23
k	0.18	0.13	-0.12	0.06
n_e	0.02	0.11	-0.20	0.08
Size 94	0.04	-0.11	-0.14	0.04
Size 95	-0.18	-0.04	-0.06	-0.18
Varroa 94	-0.16	-0.14	0.16	0.07
Varroa 95	-0.01	-0.55	0.01	-0.05
Honey 94	0.05	-0.53	-0.51	0.15
Honey 95	-0.20	-0.19	0.13	0.38
Emigration Drones	1			
Emigration Workers	0.13	1		
Immigration Drones	0.66*	-0.27	1	
Immigration Workers	-0.07	0.11	-0.51	1

Figure 2 Schematic map of the performance yard Schwarzenau from which the samples were taken. The sampled colonies are black, numbered from 1 to 38 and clustered in groups of up to five in bee shelters (rectangles) which are separated through dense vegetation of at least 18 m. According to Jay (1966, 1968) such an arrangement of hives reduces drift. The direction of the flight entrances is indicated with black bars (ellipses and irregulars = vegetation, rectangles = buildings).

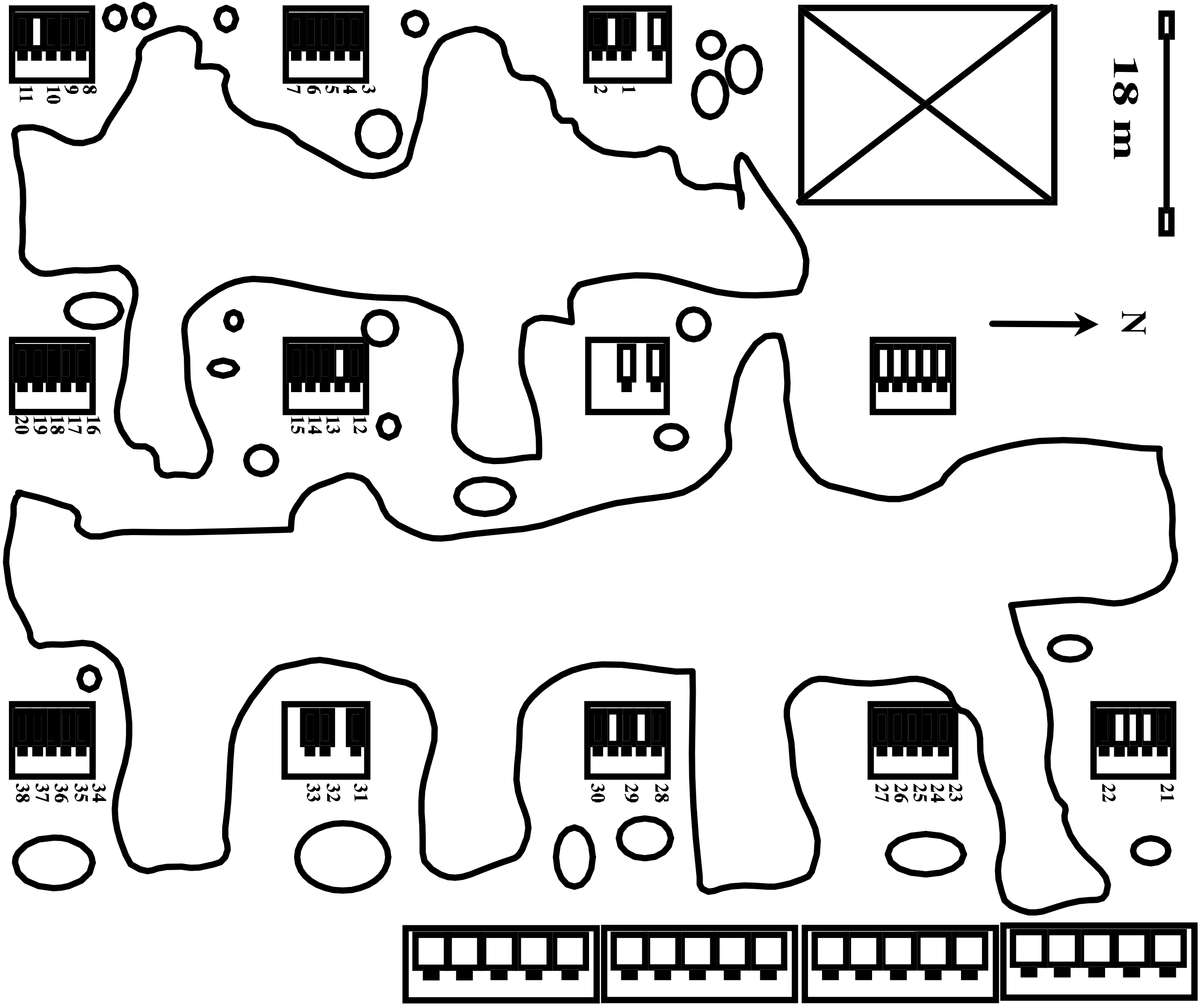


Figure 3 Immigration and emigration of workers and drones in the tested colonies. The immigration and emigration of drones was significantly higher than of workers (Mann-Whitney U-test: immigration: U = 23, p< 0.0001; emigration: U = 0; p< 0.0001).

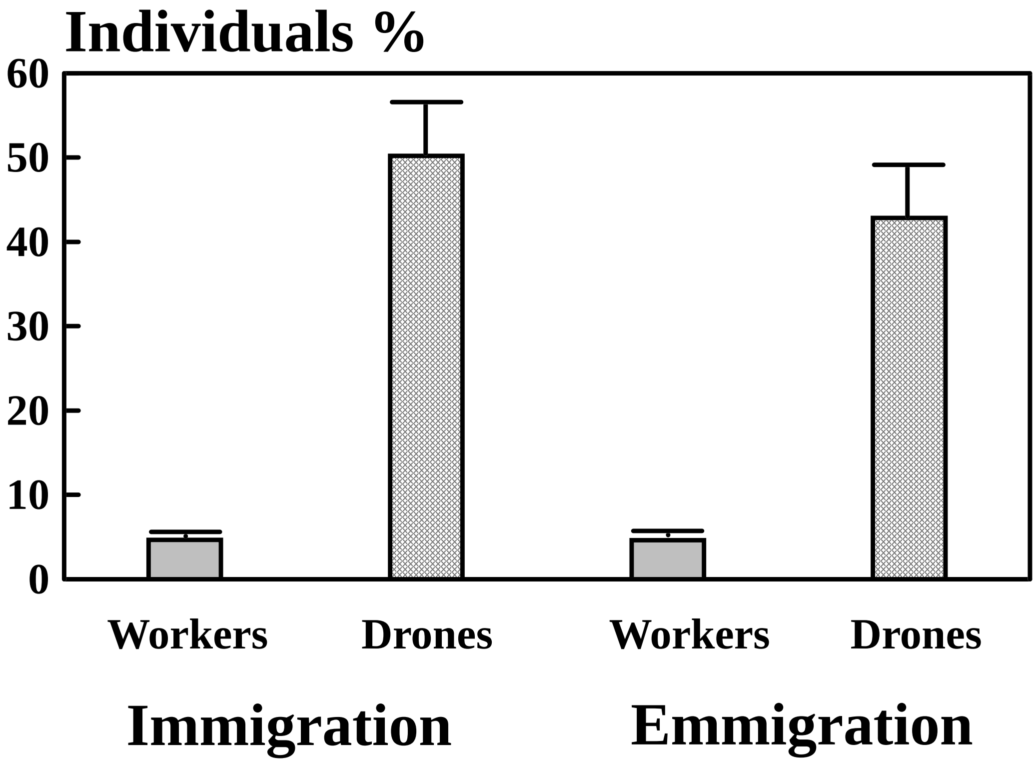
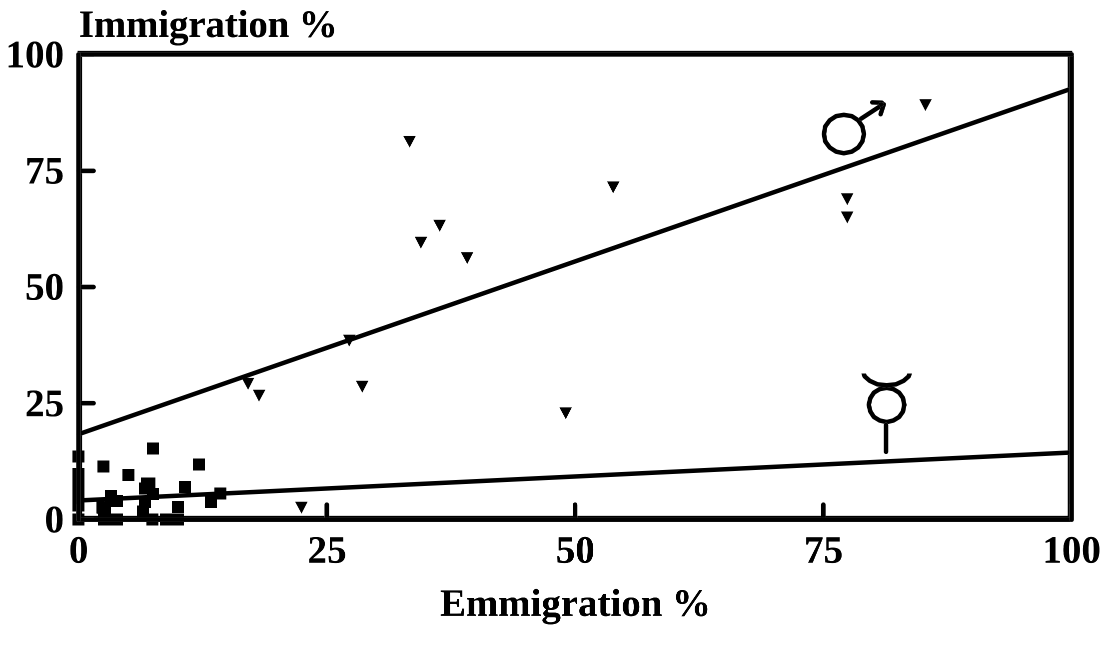


Figure 4 Adoption of foreign workers and drones. We found a significant correlation between the immigration and emigration for drones (triangles, simple correlation, r-matrix: r = 0.66, p < 0.02) but not for workers (squares, simple correlation, r-matrix: r = 0.01, p > 0.96). The two slopes were significant different from each other (distance test between the two regression coefficients, p < 0.01).



Zusammenfassung

In dieser Arbeit wurde die genotypische Komposition von Völkern der Honigbiene (*Apis mellifera*) mit Hilfe der DNA Microsatellitentechnik analysiert. Die erhobenen genetischen Daten wurden zur Analyse von zwei Verhaltensweisen verwendet, die entscheidend die Verwandtschaftsstruktur der Kolonien beeinflußen können: die Polyandrie der Königin und der Verflug von Arbeiterinnen und Drohnen. Dadurch wurde versucht, einen Beitrag zur Klärung zentraler Fragen der Evolutionsbiologie und der Bienenzucht zu leisten.

Es konnte eine hohe Variabilität der Polyandrie bei *Apis mellifera* gezeigt werden. Für natürlich gepaarte Königinnen wurde eine Variationsbreite von 10-28 Paarungen pro Königin nachgewiesen. Vermutlich können Umweltfaktoren eine entscheidende Rolle bei der Mehrfachpaarung spielen. Signifikante Unterschiede in der Paarungshäufigkeit wurden zwischen Königinnen gefunden, die sich auf Inseln ohne Drohnen (6.45 ± 4.2), auf Inseln mit Drohnen (13 ± 1.14) oder auf dem Festland gepaart hatten (18.06 ± 1.08). Für die Paarung der Honigbiene ungünstige klimatische Gegebenheiten auf den Inseln sind dabei wahrscheinlich von Bedeutung. Meine Ergebnisse für die Drohnen freie Insel zeigen, daß erfolgreiche Paarungsflüge der Königin über größere Strecken offenes Wasser möglich sind, die in Kombination mit großer Entfernung zu Drohnen produzierenden Völkern die Paarungshäufigkeit erniedrigen. Die gefundene große Varianz läßt vermuten, daß die Paarungshäufigkeit bei Honigbienen unter Umständen charakteristisch für lokale Populationen ist.

Es konnte kein signifikanter Zusammenhang zwischen dem Phänotyp der Kolonien und der Höhe der Polyandrie gefunden werden. Jedoch zeigen schwache, nicht signifikante Trends, daß die Polyandrie einen positiven Beitrag zur Produktivität auf der Ebene der Kolonie leistet, wie er von den Hypothesen zur Evolution der Mehrfachpaarung gefordert wird.

Der Verflug der Drohnen war höher als der Verflug der Arbeiterinnen. Die Ein- und Auswanderung verflogener Tiere war für Drohnen aber nicht für Arbeiterinnen korreliert. Es bestand kein Zusammenhang zwischen der Aus- oder Einwanderung von Drohnen und Arbeiterinnen einzelner Kolonien, was darauf hindeutet, daß unterschiedliche, u.U. Kasten spezifische Erkennungs- bzw. Adoptionsmechanismen von Bedeutung sind. Es konnte kein signifikanter Einfluß des Verflugs auf der Ebene des Phänotyps der Gastkolonien gefunden werden. Gleichfalls bestand kein Zusammenhang zwischen der Paarungshäufigkeit der Königin und der Höhe des Verflugs. Die Analyse zu den Modellen der Nestgenossenerkennung läßt daher vermuten, daß "foreign label rejection" bei Honigbienen unwahrscheinlich ist.

In dieser Studie wurde erstmalig die DNA Microsatelliten Technologie für Fragen der Bienenzucht angewandt. Ein Zuverlässigkeitstest wurde für eine potentielle Belegstelle auf der Insel Baltrum durchgeführt. Der Test zeigte, daß kontrollierte Paarungen auf Baltrum nicht garantiert werden können, da Paarungen der Baltrum Königinnen mit Drohnen vom Festland nachgewiesen wurden. Interaktionen mit den benachbarten Inselbelegstellen Langeoog und Norderney waren jedoch unwahrscheinlich. Die im Vergleich zu den benachbarten Belegstellen signifikant geringere Paarungshäufigkeit der Baltrum Königinnen zeigte, daß die Paarungsbedingungen auf der drohnenfreien Insel als erschwert zu werten sind. Für die Beurteilung der Sicherheit einer etablierten Belegstelle Baltrum im Routinebetrieb ist jedoch eine erneute Überprüfung mit einer ausreichenden Anzahl an Drohnenvölkern erforderlich.

Der Paarungserfolg auf den Inselbelegstellen Langeoog und Norderney, auf den Landbelegstellen Gramschatzer-Wald und Königswald und auf den Gebirgsbelegstellen Rachel-Diensthütte und Hochgrat. wurde über die Paarungshäufigkeit der Königin erfaßt. Die geringere Paarungshäufigkeit

der Königinnen auf den Inselbelegstellen war nicht auf die Anzahl der Drohnenvölker zurückzuführen.

Die Zuverlässigkeit des Prüfhofs Schwarzenau wurde getestet. Der Verflug der Arbeiterinnen war deutlich geringer als in früheren Untersuchungen von kommerziellen Bienenständen. Die Arbeiterinnen bevorzugten nicht reziprok ihre jeweiligen Heimatvölker. Der geringe Arbeiterinnen- sowie der hohe Verflug der Drohnen zeigten keinen Effekt auf die Erhebung der Leistungsdaten. Unverwandte Königinnen können jedoch die Ergebnisse verfälschen, da gezeigt wurde, daß untersuchte Prüfgruppen nicht aus Geschwisterköniginnen bestanden. Dies läßt vermuten daß Fehler bei der Königinnenaufzucht auftraten.

Für die in dieser Arbeit verwendeten DNA Microsatelliten wurde ein Zuverlässigkeitstest unter Zuhilfenahme einer künstlich besamten Königin durchgeführt. In einem Doppelblind Versuch konnten die 10 für die Besamung verwendeten Drohnen in der Arbeiterinnennachkommenschaft der Königin nachgewiesen werden. Da auch kein sichtbares Mutationsereignis gefunden wurde, scheint das bei Honigbienen angewendete Verfahren ähnlich zuverlässig zu sein wie in der forensischen Medizin.

Curriculum vitae of
Peter Neumann

PERSONAL DATA

Nationality:	German
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EDUCATION

Prim. school:	1974-1980	Robinson-Grundschule, Berlin
Highschool:	1980-1987	Lessing-Oberschule, Berlin
Abitur:	22. Jun. 1987	grade: sehr gut (A)

ACADEMIC QUALIFICATIONS

Vordiplom in Chemistry TU Berlin (18. Oct. 1988).
Vordiplom in Biology FU Berlin (14. Nov. 1991)
Diplom in Biology FU Berlin (25. Oct. 1994), grade: sehr gut (A),
Current position: Research and teaching assistant at the TU Berlin

MAJOR FIELDS OF TRAINING AND RESEARCH EXPERIENCE:

Laboratory Assistant, Institut für Humangenetik FU Berlin (1.1.1993-31.3.1993);

Teaching Student Assistant, TU Berlin (3.1993-12.1994); courses in statistics and genetics for undergraduates.

Research and Teaching Assistant, TU Berlin (DFG funded project): The role of drifting for the social parasitic strategy of laying *A. mell. capensis* workers. Principal investigator: Prof. Dr. RFA Moritz (since 1.1.1995); courses for graduates:

Laboratory: a) Molecular genetics of honeybees (1995/1996/1997), b) Population genetics of honeybees (1995/1996), c) Morphology and genetics of social insects (1997),

Field excursions: Behavioural Ecology of wood ants (1995/1996).

Temporary Visiting Researcher at the Department of Genetics (since 1.1997) and at the Ecological Research Station, Öland (5.-7.1997) of Uppsala University (DAAD funded project), Pricipial Swedish investigator: Prof. Dr. P Pamilo

International workshops: Kinship Theory (EU-TMR Network), Mols, Denmark, (1997), Social Evolution in Vertebrates and Invertebrates (ESF), Castleton, UK (1997)

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Erklärung

Sehr geehrte Damen und Herren

hiermit erkläre ich an Eides statt, daß ich für meine Promotion keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe und daß die inhaltlich oder wörtlich aus anderen Werken entnommenen Stellen als solche kenntlich gemacht wurden.

Mit freundlichen Grüßen,

Peter Neumann