variances[1]), there is a significantly lower rate for the red back strain. One of the most noticeable effects of the *Pro* locus has to do with hatchability. The trait's value is about 40% units below that of the control. a highly significant difference. In the third experiment, the effect is so large as a mean square, that it exceeds the 'between repetitions within strains' mean square term. With respect to development time, pods from the dominant strain take about 1.4 days longer to hatch than those from the control strain, a significant difference. There is no evidence that mortality is higher once the grasshoppers have emerged, that is, up to the fourth instar. From the fifth instar to 1 week post-fledging, there is a significantly higher mortality of red back insects. *M. sanguinipes* normally go through five instar stages[7]. This study shows that about ⅔ of red back individuals have an extra instar ('inserted' between the normal third and fourth instar stages); this proportion is highly significantly greater than that of the control strain. Both strains display statistically similar diurnal coupling distributions and oviposition site preferences. If one can extrapolate from these laboratory findings, it is likely that red back's rarity in nature is due, in part, to its association with low hatchability, longer hatching time and greater mortality during the later stages. Variation in the number of nymphal stages occurring prior to fledging is common in species of grasshoppers and locusts[7], including *M. sanguinipes*[8]. While temperature, nutrition, and crowding are important influencing factors[9–11], the present result (the first for an acridid) reveals a genetic connection as well. According to Pfadt et al.[8], adult weight and development time of (non-red back) 6-instar grasshoppers tend to be greater than those of 5-instar individuals (data not presented here suggest the same is true for red back variants). These features could allow a species to increase its habitat range, as appears to be the case in *M. femurrubrum*[6]. But red back is rare everywhere[4], notwithstanding a couple reports of local populations with high incidences of the morph[12]. Any benefits to red back that a supernumerary stage might accrue (perhaps in more southern areas), are obviously balanced by other factors. In northern climates, however, having an extra instar would have to be considered disadvantageous.

It could be argued that the effects detected are not due to the *Pro* locus itself but are the result of loci elsewhere in the red back stock's genome. It will be recalled, however, that the variant strain in its development received an infusion of genes from the control strain, thereby lessening the possibility of background effects, certainly those associated with loci on other chromosomes (2n ♂ = 23 in this species). Nonetheless, there may still be present in the red back strain, genes from nature that are closely linked to the *Pro* locus, and that are, in part, responsible for the effects revealed here and perhaps the trait's low incidence. The latter would require, of course, that the *Pro* locus and such genes be in linkage disequilibrium[13]. While this possibility cannot be discounted, it should be noted that thus far, population studies have revealed no evidence of this phenomenon in *M. sanguinipes* at least with respect to color and electrophoretic traits[4,14]. In any case, it cannot be unequivocally stated that the fitness disturbances are solely the result of pleiotropy on the part of the *Pro* gene.

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Intracolonial worker relationship and sperm competition in the honeybee (*Apis mellifera* L.)

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Summary. Queens, homozygous for three marker genes, were inseminated with eight different types of semen, each carrying one combination of the markers. Intracolonial worker relationship ($\hat{r}_c$) was estimated by genotype frequencies in the offspring of the experimental queens. $\hat{r}_c$ was larger than under random distribution of semen types in the spermatheca. Estimates of most quantitative genetical parameters will be more accurate using the presented estimator for intracolonial relationship. The insemination order affected genotype frequencies in the offspring, suggesting a weak 'last male advantage'. There was, however, no evidence of intra spermathecal sperm competition.

Key words. Apis mellifera; sperm competition; relatedness.

The problem of relatedness of workers in colonies of social hymenoptera and its consequences for the evolution of social behavior and the mechanisms of kin recognition, has been addressed many times since the stimulating papers of Hamilton[1,2]. In honeybees, however, polyandry weakens the arguments of kin selection as the average intracolony relationship between workers is low. Recently it has been repeatedly shown[3,4] that semen of different drones is well mixed in the spermatheca of the queen and that there is no clumping of semen as claimed by others[5,6]. However, the drones which were used for insemination did not contribute equally to the offspring, which increased the average intracolonial relationship. This has not only consequences for socio biological theories[4], but also affects important estimators in quantitative genetics. In studies which estimate heritabilities of worker characters mostly equal contributions of each drone were assumed[7,8].

The reason for an unequal contribution of drones to the offspring of a honeybee queen is unclear at present. It might be a result of male or sperm competition which could occur at three different levels. 1) There is considerable inter drone variation concerning the number of sperms per drone[9]. Therefore drones with a large number of sperms may be genetically more successful than others. 2) During insemination the semen is injected into the queens genital tract and may not be distributed at random. The queen removes this semen load slowly (up to 40 h[10]) and less than 10% of the injected semen actually enters the spermatheca[9]. This post insemination distribution therefore may affect the probability for semen to migrate into the spermatheca. 3) Intraspermathecal sperm competition also could lead to unequal fertilization frequencies of different genotypes. Fitter semen should show a higher fertilization rate than sperms with a reduced fitness.

An experiment, controlling these effects, should reveal more evidence about the origin of unequal paternity in honeybee colonies. In this study the intra colonial relationship, and the fertilization success of different semen types, will be documented with a set of three recessive marker genes.

Materials and methods. Ten queens, homozygous for three recessive phenotypical visible marker genes (cordovan (cd), diminutive (di), pearl (pe)), were artificially inseminated, according to the method of Ruttner[9], with 8 µl of semen of eight different genotypes (1 µl per genotype). Each semen type had a different combination of the above mutations. Therefore the paternity of each offspring worker produced by the inseminated queens could be doubtlessly identified by its phenotype (table 1). In order to obtain equal semen samples of all eight genotypes, 4 µl semen of each type were pooled, diluted in a salt-Tris-buffer (1.1% NaCl, 0.1% glucose, 0.01% L-arginine, 0.01% L-lysine, 0.05 M Tris (hydroxy methyl)-amino methane, pH 8.7) and centrifuged into a capillary at 10,000 x g for 10 min[3,11,12]. Equal volumes were taken from the same part of the capillary for each insemination. Semen counts in a hemocytometer[13] revealed that equal sperm numbers (average 1,482,200/µl ± 6.1%, n = 35) of each genotype were used per insemination with this procedure. Four queens, heterozygous for all three markers, were narcoticized twice with CO_2 (for 10 min within two days). The procedure induces oviposition and the queens only lay unfertilized eggs which develop into drones. This experiment should document differences in hatchability caused by the genetic markers. All queens were kept in small colonies of about 3000 worker bees. Sealed brood was placed into an incubator (35 °C, 65% relative humidity) and emerged adults were collected daily to determine the genotype frequency.

Results. Table 2 shows the results of the drone laying queens. The frequencies for all possible genotypes are not significantly different for all tested queens (G = 17.6, n.s.). Hence there was no effect of the marker genes on hatchability and no evidence for any meiotic drive mechanisms, since all marker genes segregated at random.

The total numbers of worker offspring of the inseminated queens is given in table 3. There is no homogeneous distribution because of intra queen variation ($\chi^2 = 1097.4$, df = 63, $p < 0.001$). Friedman's rank test shows that there is no significant effect of the sperm genotype on its fertilization success ($\chi^2 = 5.04$, n.s.). The daily genotype frequency counts (10 days) did not differ significantly from the total ratio given in table 3 for each queen ($\chi^2 = 238.28$, df = 639, n.s.) and there was no evidence for any semen clumping. Figure a illustrates how the 8 different semen fractions, ranked in the order of fertilization success, contribute to the offspring of each queen. The most effective semen fertilizes about 25% of all eggs laid. The least fittest semen is in less than 5% of all cases successful. Assuming large noninbred populations, the relatedness between drones and queen in natural matings should be close to zero. Therefore the average relationship of workers in colonies '$\hat{r}_c$' with natural mated queens, may be estimated according to Laidlaw and Page[4] as follows:

$$r_i = (3/4)p_i + (1/4)(1\text{-}p_i) \quad (1)$$

r_i = coefficient of average additive relationship[8] of a member of a within colony subfamily with one common father i, to the other workers in the colony

p_i = frequency of individuals with genotype i (= subfamily i)

$$\hat{r}_c = \sum_i^n r_i p_i = 0.324 \quad (2)$$

n = number of subfamilies with a standard error of the estimate SE = ±0.0083

The average intracolony dominance relationship '$\hat{r}_d$'[8] can be estimated by

$$\hat{r}_d = \sum_i^n p_i/2 = 0.086 \quad (3)$$

Figure b shows that there was a significant effect of the insemination order on the genotype frequency in the offspring. The

Table 1. Insemination pedigree of experimental colonies. Each queen was inseminated with 8 µl semen set up by 1 µl of each different genotype. The semen was sampled into one insemination syringe and injected in one session

Genotype male	Genotype female	Genotype offspring
+ + +	cd pe di / cd pe di	+ + + / cd pe di
cd + +		cd + + / cd pe di
+ pe +		+ pe + / cd pe di
+ + di		+ + di / cd pe di
cd pe +		cd pe + / cd pe di
cd + di		cd + di / cd pe di
+ pe di		+ pe di / cd pe di
cd pe di		cd pe di / cd pe di

Table 2. Genotype frequencies in drone offspring of CO_2-narcotized control queens. There is no significant difference in hatchability between the 8 different genotypes (Goodness of fit test, G = 17.6, df = 28, n.s.)

Queen	+ + +	cd + +	+ pe +	+ + di	cd pe +	cd + di	+ pe di	cd pe di
1	20	18	17	19	20	28	24	21
2	18	15	12	13	14	11	16	15
3	19	15	13	23	16	22	15	14
4	17	18	20	12	21	17	17	11
Total	74	66	62	67	71	78	72	61

Table 3. Numbers and genotype of worker offspring in artificial inseminated queens. The frequencies are significantly different between queens, but there is no evidence for genetically determined differences in semen fitness. The order of insemination is given behind each offspring value (from **1** = first semen fraction to **8** = last semen fraction for each queen)

Queen	+ + +	cd + +	+ pe +	+ + di	cd pe +	cd + di	+ pe di	cd pe di
1	50 **2**	72 **7**	3 **6**	73 **5**	44 **8**	16 **1**	43 **3**	27 **4**
2	99 **7**	111 **6**	95 **4**	55 **5**	73 **2**	31 **3**	24 **1**	75 **8**
3	24 **8**	10 **4**	8 **1**	9 **5**	12 **3**	44 **7**	23 **6**	91 **2**
4	34 **8**	21 **6**	33 **5**	51 **7**	18 **2**	9 **3**	16 **4**	8 **1**
5	22 **3**	36 **4**	36 **5**	61 **8**	112 **6**	126 **7**	29 **1**	55 **2**
6	12 **1**	19 **6**	15 **4**	22 **7**	24 **2**	54 **8**	37 **5**	40 **3**
7	79 **7**	115 **6**	62 **5**	63 **4**	38 **3**	32 **1**	19 **2**	119 **8**
8	48 **8**	5 **4**	16 **2**	45 **5**	8 **1**	33 **6**	20 **7**	24 **3**
9	68 **6**	99 **8**	105 **5**	75 **2**	62 **1**	38 **4**	93 **7**	21 **3**
10	23 **3**	15 **5**	16 **8**	59 **6**	26 **1**	9 **2**	50 **4**	108 **7**
Total	459	503	389	513	417	392	354	568

first semen portion in the insemination syringe contributed very little, whereas the according genotype frequency increased with increasing rank in insemination order. Page's L-test[14] reveals a significant positive trend (L = 1837.5; p = 0.01).

Discussion. The present results agree with previous observations that semen in the spermatheca is well mixed[3,4,15-17]. Reports of clumping of semen[5,6] could not be supported. The average intracolonial relationship estimated in this study is low. In species or subspecies which are highly polyandrous, such as *Apis cerana*[18] or the Africanized honeybee in South America[19], natural matings of queens may result in intracolonial relationships which are close to 0.025. For European *A.mellifera,* where queens mate 8–10 times[20-22], $\hat{r}_c$ may be larger, because of unequal frequencies of semen in the spermatheca. The effect of nonrandom semen contribution also affects estimates of heritabilities (h^2) for worker characters in sib analysis. Published values for h^2 therefore may be slightly overestimated[23-26] as most authors assume equal semen distribution. Based on Falconer's equation[27] a more accurate estimate may be given by

$$h^2 = T/\hat{r}_c = (1/\hat{r}_c)Cov_W/Var_p = 3.09\ Cov_W/Var_p \quad (4)$$

with h^2 = estimate for heritability
$\hat{r}_c$ = average intracolonial relationship
t = intraclass correlation
Cov_W = covariance between workers within a colony
Var_p = Phenotypic variance of the according character

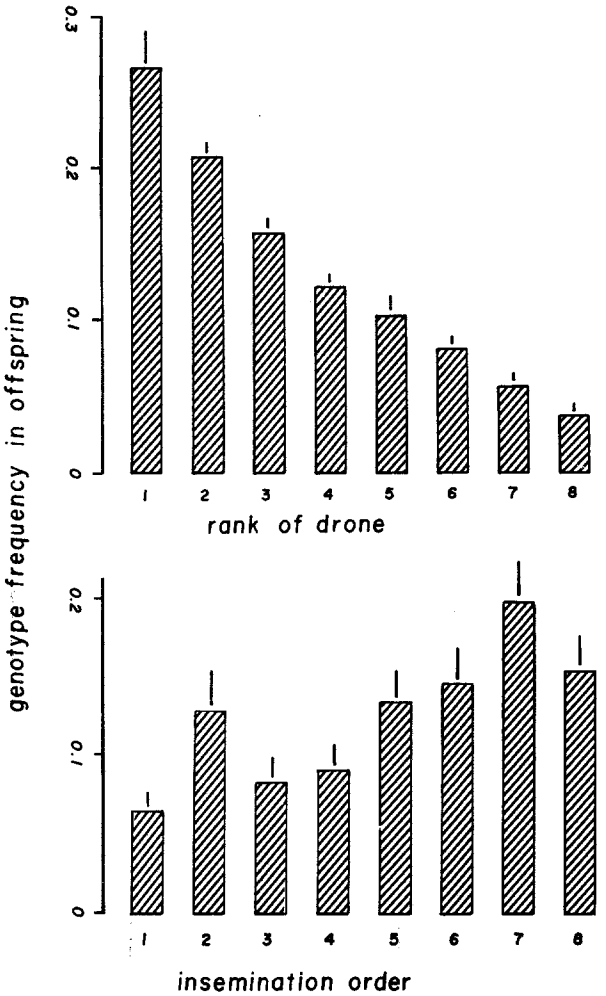
which regards the average relationship as estimated in (1). There may be a bais of this estimate due to dominance variance of $0.264\ Var_D/Var_p$ (with Var_D = dominance variance). When all drones, used for insemination, originate from one 'sire-queen', a common procedure in honeybee breeding, $\hat{r}_c$ can be estimated by 0.5506 and $\hat{r}_d$ equals 0.272. This results in a h^2 – estimate for worker characters of

$$h^2 = 1.82\ Cov_W/Var_p \quad (5)$$

with a bias of $0.459\ Var_D/Var_p$ due to dominance effects.

The estimate for the average worker relationship in matings with unrelated drones is still considerably smaller than for full sibs in case of single mating in both, male-diploid (r = 0.5) and male-haploid (r = 0.75) populations. Therefore kin selection may only play a minor role in honeybee populations. Since there is no sperm clumping in the spermatheca[3,4,28] there is no evidence of temporary numerical superiority of certain kin groups, which might support kin selection hypothesis.

Although sperm competition is a frequent feature in polyandrous insects[29], there is no evidence of intraspermathecal sperm competition in honeybees. However the insemination sequence did lead to extra spermathecal sperm competition. More proximal semen fractions in the syringe were genetically more successful than distal fractions. This phenomenon might fit to a type of 'last male advantage'[29] for a single semen injection, which was, however, not as strong as observed in other insects[30-32]. The last male was about twice as successful as the first one and intermediate males showed increasing fertilization success the higher their rank in insemination order. Similar results were observed by Woyke[33], who tested four queens in a two years study. He found the first male to be half as effective as the last male. However the first male was more effective than males with



a The average proportion of offspring (±SE) produced by eight different drones. The drones are ranked from 1 to 8 (successful to unsuccessful), *b* The average frequency dependent on the rank in insemination order. There is a significant increase in fertilization success with increasing insemination rank (Page's L-test; L = 1873.5 p = 0.01).

intermediate ranks of insemination order. Laidlaw and Page[4] did not observe any effect of the insemination order in their recent study and hence concluded, that there should be no sperm precedence or depletion in honeybees.

One explanation for those contradictory results from previous studies might be the uncontrolled number of sperms used for each insemination. Number of sperms were not counted or standardized prior to insemination in both studies which did use undiluted semen of drones. Therefore inter male sperm number variation may have covered the effect of extra spermathecal sperm competition. Another reason might be differences in insemination technique, which may well affect the distribution of semen in the vagina and oviducts prior to entering the spermatheca. A nonrandom distribution correlating to the packing in the syringe should be most likely. As the migration of spermatozoa into the spermatheca is a function of time[10,27], those fractions close to the spermathecal duct therefore might enter in higher numbers than semen located in the lateral oviducts. This post insemination sperm distribution may be strongly affected by the techniques used. Therefore also comparisons to natural matings should be made carefully. Up to now we do not know whether there is any sperm competition in natural matings. The present data may suggest such mechanisms but definitely more experimental data on natural matings is neccessary to rule out other hypotheses.

Nevertheless there is some evidence in the mating behavior of drones which may have evolved from 'last male advantages'. The mating sign, a part of the male endophallus, is left in the queens bursa copulatrix[34] after mating. Though at present its function during mating is unclear, it may well have evolved from a type of 'mating plug' to prevent other males from mating[29]. However in natural matings this mating plug apparently is not very efficient. The honeybee is highly polyandrous and *A. cerana* queens can mate with up to 30 males[18]. During mating drones can easily remove the mating sign of the previous one[35]. Therefore other strong selective forces at the queen or colony level are likely to counteract the desired 'last male strategy' of the drone. A selective advantage for highly polyandrous queens could be an efficient counter strategy. There are several studies and hypotheses showing that multiple mating of the queen may indeed increase individual–, colony–, and population – fitness in honeybees[36–38].

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Influence of host plant and larval diet on ovarian productivity in *Acrolepiopsis assectella* Z. (Lepidoptera: Acrolepiidae)

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Summary. Females of *Acrolepiopsis assectella*, reared on a semi-synthetic diet and laying on artificial substrates, do not respond to external stimuli by increasing ovarian production. When returned to the natural host (*Allium porrum*) for only one generation, ovarian production again rises and reaches the same level as in wild females, but its variability is strongly reduced. We conclude that selection under artificial conditions eliminates individuals which strictly depend on host plants for stimulation of larval nutrition and of reproduction.

Key words. Lepidoptera; *Acrolepiopsis assectella*; host plant; larval diet; ovarian production; stimulation; insemination.

Oogenesis in most insect species is related to environmental signals, and reserves stored during the feeding period. Stimulation of oogenesis is under the control of the brain and is mediated by the endocrine system, as has been shown for example in *Drosophila*[1]. In many phytophagous Lepidoptera, reserves are accumulated during larval life. Adults have no feeding needs;