Male behavioural maturation rate responds to selection on pollen hoarding in honeybees

By: OLAV RUEPPELL, ROBERT E. PAGE, JR, and M. KIM FONDRK

***Note: Figures may be missing from this format of the document

Abstract:

Division of labour in social insect colonies relies on behavioural functional differentiation (specialization) of individuals with similar genomes. However, individual behavioural traits do not evolve independently of each other (behavioural syndromes). A prime example is the suite of behavioural differences in honeybee workers that has evolved in response to bidirectional selection on pollen hoarding of honeybee colonies (pollen-hoarding syndrome). More generally, these differences reflect functional differentiation between nectar and pollen foragers. We demonstrate here that this pollen-hoarding syndrome extends to drones. Similar to what has been shown in workers, drones from the high-pollen-hoarding strain had a higher locomotion activity after emergence, and they initiated flight earlier than did males derived from the low-pollen-hoarding strain, with hybrids intermediate. However, these two behavioural traits were unlinked at the individual level. We also found that social environment (the colony) affects the age at which drones initiate flight. The indirect selection responses of male behaviour suggest that male and worker evolution are not independent and may constrain each other's evolution. Furthermore, we identified three distinct peaks in the probability of flight initiation over the course of the experiment and a decreased phenotypic variability in the 'hybrid' males, contrary to quantitative genetic expectations.

Article:

Social insects owe much of their evolutionary success to the coordinated action of behaviourally specialized members of their colonies (Oster &Wilson 1978; Winston 1987; Hölldobler &Wilson 1990). Division of labour is a hallmark of social evolution (Beshers & Fewell 2001), and among honeybee workers, it is mainly determined by age and genetic effects (Winston 1987). To promote individual specialization, the response thresholds to different task stimuli should vary independently among individuals (Beshers & Fewell2001). This seems not to be the case: honeybees show well-established behavioural differences among races that extend to multiple behavioural phenotypes, as well as to morphology and life history (Winston 1987). Similar multiple behavioural phenotypes in honeybees are also correlated at the individual level (Page & Erber 2002; Pankiw 2003; Scheiner et al. 2004), indicating their evolution as behavioural syndromes (array of correlated behavioural characteristics: Sih et al. 2004). Linked sets of behavioural traits may have arisen through adaptation in certain contexts (Amdam et al. 2004), but the correlation among behavioural traits may constrain the division of labour in social insects, and behavioural optimization in general (Price & Langen 1992; Sih et al. 2004).

One of the best-studied examples of behavioural syndromes in insects is the behavioural differentiation among workers of two strains of honeybees that have been bidirectionally selected over multiple generations for different amounts of pollen stored in their nests (Page & Fondrk 1995). Many different aspects of foraging behaviour (Page & Fondrk 1995; Page et al. 1995, 1998; Waddington et al. 1998; Pankiw &Page 1999; Fewell &Page 2000) have changed in concert with seemingly unrelated behavioural phenotypes, such as behavioural maturation (Calderone &Page 1996; Pankiw &Page 2001) and learning behaviour (Scheiner et al. 2001a, b). This suite of behavioural correlations is also reflected in racial differences and in unselected honeybee workers (Pankiw & Page 2000; Pankiw 2003), and thus it constitutes a general behavioural syndrome (Sih et al. 2004).

In contrast to the amount of work investigating the relations between behavioural phenotypes within honeybee workers, correlations across castes or sexes have not been addressed (but see Giray & Robinson 1996), even though male–female correlations are of general importance to understand the evolution of animal behaviour (Price & Langen 1992; McGlothlin et al. 2004). Male honeybees (drones) serve an exclusively reproductive function. They mature for up to a week in the hive without performing hive duties before initiating mating flight activity (Ruttner 1966). Mating flights are concentrated around midday (Drescher 1969). Drones are selected for fast maturation to maximize their chances of finding and mating with a queen during repeated mating flights (Page 1981). Conversely, the maturation of workers from hive bees to foragers is flexible and is regulated by a number of factors, because workers fulfil important colony functions in the hive as well as during foraging (Winston 1987). As a result of their different colony functions, drones differ substantially from their sister workers in many respects, including morphology, development and physiology (Winston 1987).

Despite these differences, juvenile hormone seems to affect the adult maturation rate similarly in drones and workers (Giray &Robinson 1996; Tozetto et al. 1997). Furthermore, Giray &Robinson (1996) found a general correlation between drone and worker maturation by comparing the age at first flight of drones derived from colonies that differed in their tendency to produce precocious foragers. Thus, similar regulatory circuits may govern worker and drone adult development. We addressed in this study whether the pleiotropic associations of the pollen-hoarding syndrome in workers can be extended to the maturation of drones.

We measured two different behavioural parameters ('initial locomotor activity' and the 'age at the onset of flight') to compare the maturity of high-pollen-hoarding drones, low-pollen-hoarding drones, and the F1 hybrids. The age at the onset of flight (time from emergence to flight initiation) reflects the rate of adult development and is a central trait in the pollen-hoarding syndrome in workers with direct and genetic correlations to other traits (Pankiw 2003; Rueppell et al. 2004). A new experimental set-up allowed us to study the age at the onset of flight in drones on a large scale. Locomotor activity is in general also believed to be a measure of maturation rate (Muir 2000). Locomotor activity directly after emergence reflects the maturation process prior to emergence. We chose to measure the initial locomotor activity as walking activity under ambient light and under dark conditions to account for effects of possible sensory differences between the selected strains (Pankiw &Page 1999).

METHODS

Materials

The high- and low-pollen-hoarding strains used in the experiments were initially established in 1990 (Page & Fondrk 1995) and had been selected for their respective colony phenotype for 20 generations at the initiation of our experiment. High, Low and Hybrid queens were reared as sources for drones from two inbred lines representing the high- and low-pollen-hoarding strains (hybrid queens were produced by inseminating high-line queens with drones from the low line). Queens were allowed to mate naturally, and they were introduced into host colonies of similar sizes. Drones are haploid, derived directly from unfertilized eggs, and contain recombined genomes derived from their mother queen. We performed two independent sets of experiments in April and May 2002.

Experiment 1

In the first experiment, we caged all queens over 4 days on empty drone comb to induce maximal, synchronized drone egg laying. Based on the amount of brood produced, we chose one Hybrid queen and two High queens and two Low queens as sources and transferred the mature drone pupae to incubators, 1 day before emergence. The selection lines are inbred (Rueppell et al. 2004) and therefore have reduced genetic heterogeneity, so we pooled the offspring of both High queens and the offspring of both Low queens. Emerging drones were continuously collected for 2 days (approximately 1600 High, 1500 Low and 2500 Hybrid) and marked with coloured paint (Testors, Rockford, Illinois, U.S.A.) to indicate emergence date and queen source. Immediately after handling, drones were transferred to a common comb and later introduced into two large host colonies (wildtype; 40 000 bees) in which most of the pre-existing drones had been culled to minimize drone rejection (Free & Williams 1975).

The host hives were modified to allow the monitoring of flight activity of drones by daily censuses in the evening (Fig. 1). Two hive bodies containing the brood nest and the main part of the colony were placed on top of a medium-depth (16.6 cm) hive body, separated by a queen excluder that proved in preliminary experiments impenetrable to our drones. Drones were introduced into the upper part of the colony, which had three one- way exits. Drones could leave the hive through these but were forced to re-enter the hive through the regular entrance leading into the bottom hive body (Fig. 1), where
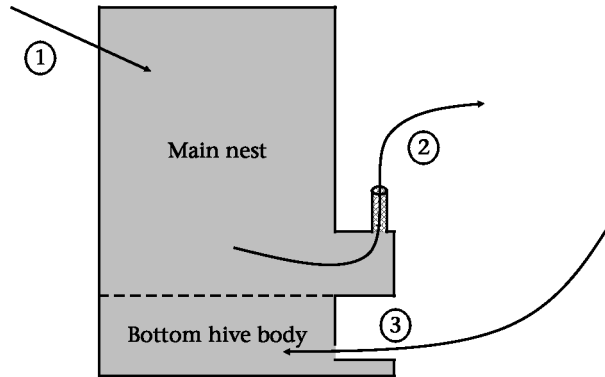
Figure 1. Host colonies for measuring the age of first flight in honey-bee drones were housed in this modified bee hive, which allowed monitoring of flight activity by a daily census of the bottom box. ① Drones were introduced into the main hive that contained the brood nest to mature. ② Once mature, drones left the hive through wire-mesh one-way exits. ③ Upon return to the hive, drones were 'forced' to enter through the main entrance into the bottom hive body, where a census was performed at the end of each day.

they accumulated on the combs until the evening census. At the end of each census, the drones were placed back into the main part of the hive.

Standard climate data (daily average wind speed; precipitation; relative air humidity; minimum, maximum and average air temperature, and soil temperature) were obtained from the California CIMIS database (http:// www.cimis.water.ca.gov) for monitoring station Number 6 (Davis), which was approximately 2 km from the experimental hives under similar local conditions. Hives were housed (facing eastwards) on an open field with rural, marginal vegetation.

Experiment 2
A second experiment was performed as described above with drones from the same queens. However, we measured locomotion activity and tagged each drone in this experiment individually before the initial introduction to a single host colony. This only allowed for relatively small sample sizes (57 High, 55 Low and 100 Hybrid). Within 10 min of emergence, each drone was scored for the following two measurements of locomotion activity. (1) Drones were transferred into a 14-cm, polystyrene petri dish that was placed on a sheet of paper with a grid drawn on it (grid width = 1.5 cm). We let each drone adjust to the experimental arena for 1 min before measuring his locomotion activity in ambient laboratory light. We recorded the number of grid crossings per 2 min as the number of grid marks (lines) completely crossed by the head of the drone. Time that drones took to climb on the petri dish walls or to right themselves after falling over were excluded from the analysis. Repeated grid crossings that resulted from immediate reversal of direction were only counted as one grid-crossing event. (2) The dish on the grid was then transferred into a completely dark room, and the drone's grid crossings were recorded as described above for 2 min through an infrared video camera (with infrared illumination). The observers did not know the source of each drone while scoring grid crossings.

Statistics

Even though most of our data differed significantly from normality, we used parametric test statistics throughout this study to allow for regression analyses and ANOVAs with standard post hoc tests. We justify this with our large sample sizes (Kallenberg 1997) and even in the smaller, second experiment the results of nonparametric tests (where possible) were consistent with the reported results of the parametric procedures. We measured group differences of age at first flight and locomotion scores with ANOVAs followed by Dunnett's C post hoc tests because variances differed between groups. For individual groups, mean f standard deviations are given. Data distribution was compared between groups with independent two-sample Kolmogorov–Smirnov tests after adjustment of the means (significance values are given after Bonferroni correction for multiple comparisons). For each phenotypic trait, we evaluated the proportion of the phenotypic variance that was attributable to the genetic differences between the experimental groups by regression analysis: age at first flight and the two walking scores were regressed on genotype. The genotypic values of Low, Hybrid and High drones were coded as 1, 2 and 3, respectively. The r2 value of such a regression is equivalent to a (broad-sense) heritability estimate at the population level because r2 equals the ratio of genotypic variance to total variance when the groups are measured in a common environment (Falconer &Mackay 1996). The genotypic coding of Hybrid drones as two is a simplification, because individuals differ from the mean genotypic value in this group. Thus, the r2 values including the hybrid drones represent alower estimate of the proportion of variance that is attributable to genetic factors. Confidence intervals for the coefficients of variation (CV) were determined by jackknifing (Manly 1997).

RESULTS

Experiment 1

A two-way ANOVA revealed that the drones from the three different genetic sources differed in the age at first flight ($F_{2,4944} = 94.8$, $P < 0.001$; Fig. 2). However, the host colony in which the drones matured also had a significant effect ($F_{1,4944} = 250.9$, $P < 0.001$) and there was a significant interaction between the two factors ($F_{2,4944} = 9.6$, $P < 0.001$; Fig. 3). Post hoc tests for the first colony revealed that High (8.1f 3.4 days) and Hybrid (8.1f 2.9 days) drones initiated flight at significantly younger ages than did Low drones (9.4f 3.7 days). In contrast, all genotypic groups were significantly different from each other in the second colony (High: 6.5f 1.3 days; Hybrid: 7.2f 1.7 days; Low: 8.0f 2.5 days). The corresponding regression analyses based on all three groups resulted in an r2 value of 0.02 for the first colony ($F_{1,2578} = 43.6$, $P < 0.001$) and 0.08 for the second colony ($F_{1,2368} = 196.4$, $P < 0.001$). Regression analyses based only on the two parental High and Low lines resulted in r2 values of 0.03 ($F_{1,1343} = 43.6$, $P < 0.001$) and 0.12 ($F_{1,1278} = 170.0$, $P < 0.001$), respectively.

Overall, the experimental groups differed not only in the mean but also in the distribution of age at first flight: Low differed from Hybrid drones (Kolmogorov–Smirnov test: Z = 5.3, $P < 0.001$), Hybrid from High drones (Z = 5.2, $P < 0.001$) and Low from High drones (Z = 8.2, $P < 0.001$). The variation was lowest in Hybrid drones (CV = 31.8%; 95% CI: 29.4–34.1), and Low drones (CV = 36.6%; 33.8–39.1) and High drones (CV = 38.8%; 35.3–41.9) were similar. An age-specific analysis of the probability of initiating flight (Fig. 4) showed that all three groups had a similar pattern of flight initiation with three peaks. Bootstrap analysis (Manly 1997) demonstrated that the age-specific flight probability in the three groups was more similar than

expected by chance (P < 0.001). However, the exact timing, shape and height of these peaks differed between groups.

Results of correlation analyses of overall flight initiation probability and various climate variables suggested little
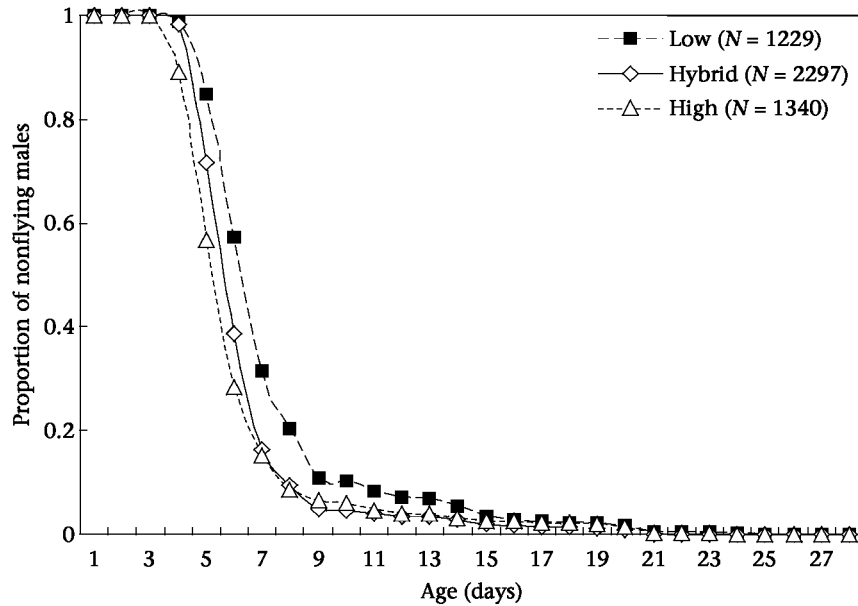

Figure 2. Onset of flight activity for honeybee drones in the Hybrid, High and Low groups in experiment 1.

influence of weather over the experimental period Pearson correlations, uncorrected for multiple independent tests: daily average wind speed: $r_{20} = 0.26$, P = 0.269; precipitation: r_{20} = -0.30, P = 0.183; relative humidity: r_{20} = -0.34, P = 0.121; minimum air temperature: r_{20} = -0.19, P = 0.404; maximum air temperature: r_{20} = 0.22, P = 0.332; average: r_{20} = 0.33, P = 0.139; soil temperature: r_{20}=0.33,P=0.135.
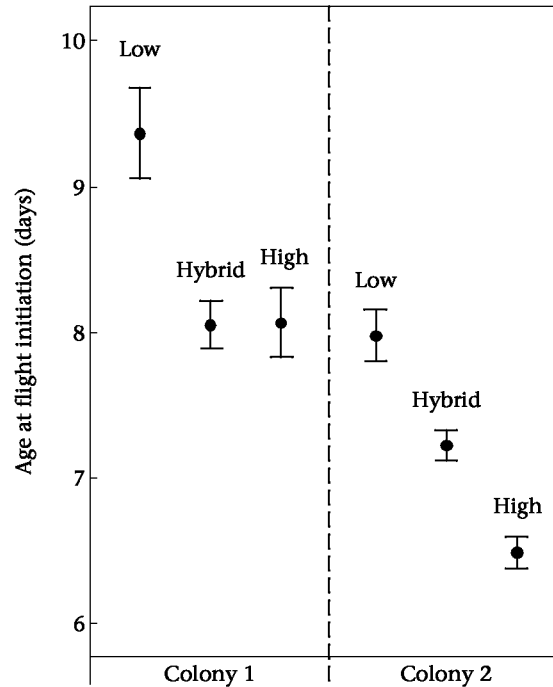
Figure 3. Effects of social environment on age at first flight in honey-bee drones in the Hybrid, High and Low groups in experiment 1. Means are given with 95% CI.

Experiment 2

The ages at first flight of the three genotypic groups in this smaller data set were also significantly different ($F_{2,139} = 8.4$, $P < 0.001$; Fig. 5), but only the differences between Low (9.6f 2.3 days) and Hybrid (8.0f 1.8 days) and Low and High (8.0f 2.2 days) drones were significant. Linear regression yielded an $_r2$ value of 0.08 ($F_{1,140} = 11.4$, $P = 0.001$) based on all groups and an r^2 value of 0.12 ($F_{1,82} = 10.7$, $P = 0.002$) based on the two selected lines only. A meaningful analysis of the age-specific probability of foraging was precluded by small sample size, but the variation was again lowest in Hybrid drones (CV = 22.0%; 95% CI: 18.0–24.6), then Low (CV = 23.4%; 20.3–25.8) and High drones (CV = 27.2%; 23.0–29.8).

A two-way ANOVA did not reveal a significant interaction between drone groups (Low, Hybrid, High) and experiments (1, 2) ($F_{2,5002} = 1.8$, $P = 0.158$). Independently, both factors (drone group: $F_{2,5002} = 21.8$, $P < 0.001$; experiment: $F_{1,5002} = 116.9$, $P < 0.001$) had a highly significant effect. The difference between the experiments in the age at first flight was in part due to adverse flight conditions during 2 days (9,10: Fig. 5) in the second experiment. However, a correctionforthese 2 days did not significantly affect any analysis within the second experiment and, therefore, only results of the uncorrected analyses are reported.

Age at first flight was neither correlated with the initial locomotion activity of the drones in the light (Pearson correlation: $r_{140} = -0.08$, $P = 0.319$) nor in the dark ($r_{139} = 0.06$, $P = 0.462$), but the activity of drones in the two assays was correlated ($r_{209} = 0.69$, $P < 0.001$). This correlation was apparent in all groups (Low: $r_{53} = 0.43$, $P = 0.001$; Hybrid: $r_{98} = 0.72$, $P < 0.001$; High: $r_{55} = 0.76$, $P < 0.001$). Overall, activity was significantly higher in the dark locomotion assay than in the light one (paired t test: $t_{209} = 8.1$, $P < 0.001$; Fig. 6). Locomotor activity differed significantly between drone groups in the light
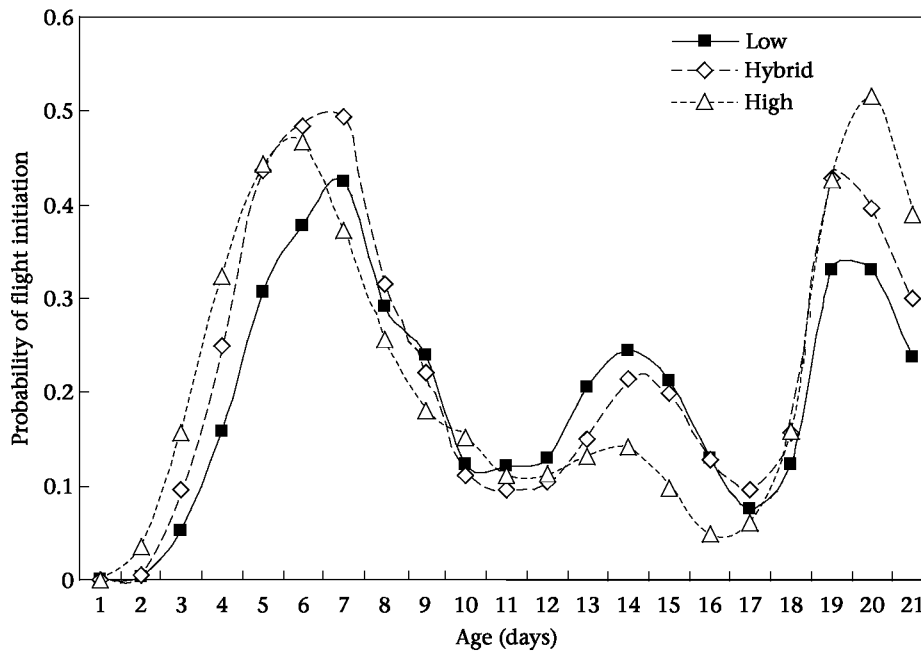
Figure 4. Age-specific probability of flight initiation in Low ($N = 1229$), Hybrid ($N = 2297$) and High ($N = 1340$) males in experiment 1. Data were smoothed (moving average, 3-day window) and truncated at 21 days of age because the low remaining sample size (fewer than 15 individuals in each group) made the estimates unreliable.

($F_{2,209} = 10.8$, $P < 0.001$) and in the dark ($F_{2,208} = 3.8$, $P = 0.023$) (Fig. 6), primarily because, as revealed by post hoc tests, Low drones walked significantly less than Hybrid and High drones. In the dark, Low drones also walked least, but the difference was significant only compared to High drones (Fig. 6). The r^2 estimates based on all groups were 0.06 ($F_{1,210} = 13.0$, $P < 0.001$) for activity in the light and 0.03 ($F_{1,209} = 7.1$, $P = 0.009$) for activity in the dark. Based only on the selected strains, these estimates were 0.13 ($F_{1,110} = 16.0$, $P < 0.001$) and 0.07

($F_{1,109} = 7.6$, $P = 0.007$), respectively. The coefficient of variation of the locomotion scores decreased from Low to Hybrid to High (in the light: Low: 125.1%; 95% CI: 86.7–153.9; Hybrid: 76.4%; 59.4–86.7; High: 70.6%; 49.0–88.1; in the dark: Low: 90.4%; 71.6–112.4; Hybrid: 76.6%; 64.5–89.0; High: 68.4%; 51.8–86.3).

Locomotion activity of drones was significantly correlated with the time of day that assays were performed (light: $r^2 10 = -0.16$, $P = 0.017$; dark: $r_{209} = -0.23$, $P = 0.001$). Therefore, we repeated the above statistical tests
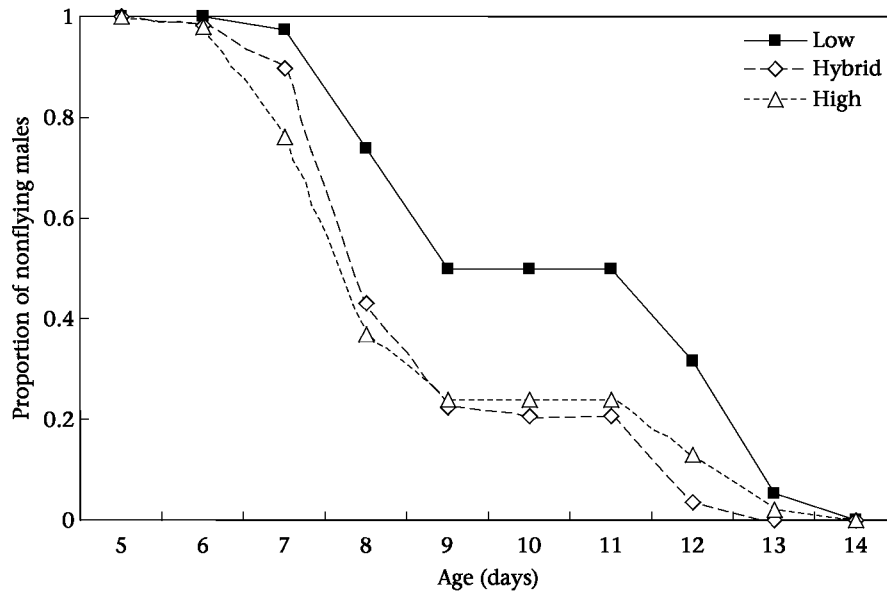
Figure 5. Onset of flight activity for honeybee drones in the Hybrid, High and Low groups in experiment 2. The absence of any drones initiating flight on days 9 (19 May 2002) and 10 (20 May 2002) was due to rain.
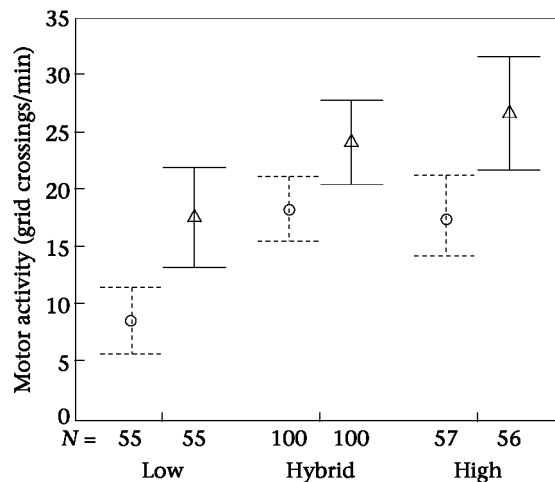

Figure 6. Differences in initial locomotor activity of honeybee drones in a walking assay in the light (○) and the dark (△). Means are given with 95% CI.

on locomotion activity with the unstandardized residuals of a linear regression on time of day; this procedure eliminates effects that are caused by the time of day when the individual drones were tested. The correlations between variables were not affected by the time correction. However, the differentiation between groups was significantly weakened (light: $F_{2,209} = 9.6$, $P < 0.001$; dark: $F_{2,208} = 2.7$, $P = 0.068$) because the emergence pattern of drones dictated a nonrandom testing order. On average, High drones were tested earlier and Low drones later, with Hybrids intermediate ($r_{210} = -0.15$, $P = 0.027$). The adjusted r^2 estimates based on all groups were 0.05 ($F_{1,210} = 10.7$, $P = 0.001$) for locomotion activity in the light and 0.02 ($F_{1,209} = 4.9$, $P = 0.028$) for activity in the dark. Based only on the selected strains, these estimates were 0.11 ($F_{1,110} = 13.2$, $P < 0.001$) and 0.05 ($F_{1,109} = 5.4$, $P = 0.023$), respectively.

DISCUSSION
The results of this study show that the behavioural maturation of honeybee drones has been changed by artificial selection on colony-level pollen-hoarding behaviour (Page & Fondrk 1995) in a way similar to worker maturation (Rueppell et al. 2004; Humphries et al. 2005). These correlations suggest that the pollen-hoarding behavioural syndrome extends from honeybee workers, where it may have evolved (Amdam et al. 2004), to drones. The described phenotypic correlations could be caused by pleiotropic genes, gene clusters or genetic drift. We favour the first explanation because our breeding scheme (circular breeding of substrains and repeated outcrossings) of the selection lines in combination with the high genomic recombination rate in honeybees (Gadau et al. 2000) makes phenotypic correlations due to genetic linkage or drift less likely.

The extension of the pollen-hoarding syndrome to drone behavioural phenotypes is important for several reasons. First, it is informative for the understanding of behavioural evolution in the social context of honeybees. Our results support the suggestion that drone and worker maturation have common underlying causes (Giray & Robinson 1996). This is not a trivial finding, because the onset of foraging activity in workers is a life history parameter that is highly regulated at the individual and colony level (Huang &Robinson 1996; Pankiw et al. 1998; Robinson &Huang 1998; Beshers & Fewell 2001; Robinson 2002; Amdam & Omholt 2003). In contrast, no social regulation of drone flight initiation has been demonstrated. Drones should be selected for rapid growth and maturation to maximize their mating chances (Page 1981; Currie 1987; Berg et al. 1997). Temporal division of labour is a key outcome of the behavioural evolution of honeybee workers, but its optimization may be impeded through counteracting selection on correlated drone traits. Likewise, drone development may be restrained by worker evolution. No studies addressing the optimal maturation rates in workers or drones have been conducted, although experimental alterations of developmental rates with manipulations of juvenile hormone titres are feasible (Giray & Robinson 1996; Sullivan et al. 2000).

Second, drones will be an important tool for pursuing more detailed studies on the proximate cause of the pollen-hoarding behavioural syndrome. Their haploid genome will facilitate analyses of the underlying genetic architecture (Giray &Robinson 1996), because dominance effects do not exist and dominant genetic markers can be used for genomic mapping. Furthermore, the drones' larger size facilitates the dissection of different organs for localized gene expression studies. Finally, the regulation of drone flight initiation is presumably less complex, and consequently drones may provide a simpler working model than workers to address the causes of different behavioural maturation rates.

Third, the trait correlations between workers and drones may represent an example of intersexual genetic correlations that is of general interest to biology (West-Eberhard 2003). Trait correlations between males and females severely influence the evolutionary dynamics of the traits (Price & Langen 1992; Roff 2002). They have been explored mainly in the field of sexual selection (e.g. Clotfelter et al. 2004; McGlothlin et al. 2004), but they are probably important inother contexts of functional differentiation of males and females (e.g. Temeles et al. 2000). Honeybee males and workers represent a prominent case of functional differentiation (Winston 1987), but our results support the hypothesis that they share common mechanisms of life history and behavioural regulation (Giray &Robinson 1996). It will be of general interest to explore the

possibility of evolutionary constraints by intersexual correlation with respect to social evolution and division of labour. The pollen-hoarding strains offer an amenable model to study at the organismal and molecular level.

Both behavioural measures, the age of flight initiation and the locomotion after emergence, differed between high- and low-pollen-hoarding drones in the same direction as in workers. However, we found no significant correlation between the two measures at the individual level. This indicates that the pollen-hoarding syndrome involves different, probably interacting factors. If the main genetic determinants of age at first flight and locomotion after emergence were identical, a good correlation between the behavioural measures would be expected, particularly in the genetically segregating hybrid drones. Despite an overall correlation, the results of the locomotion assay also differed in the light and dark. The interstrain differences were more pronounced in the light. Furthermore, the differences between groups support an additive model of inheritance in the dark (as in the age of flight initiation), but the high-strain genotype seems to be dominant for the locomotion assay under ambient light. Candidate gene analyses (Humphries et al.2003) suggest that cAMP-dependent signal-transduction cascades in the central nervous system are involved in the pollen-hoarding syndrome (Page & Erber 2002; Humphries et al. 2003). These cascades are important in a variety of contexts (Antoni 2000; Jordan et al. 2000) and the underlying genes are good candidates for wide-ranging pleiotropic effects. cAMP signalling has been specifically implicated in maturation of the nervous system (Fujioka et al. 2004) and thus may be responsible for at least one of the observed differences between drones of the high- and low-pollen-hoarding strains.

The average flight initiation time of our drones is in relatively good agreement with previous records from different times (Howell & Usinger 1933) and places (Drescher 1969). Drone flight initiation in this study also occurred over a very narrow window of time (>90% of the drones initiated flight within 5 days) and showed a lower genetic variance component (r^2 values) compared with that of workers (Rueppell et al. 2004). These findings suggest that the onset of flight in drones has been under strong, unimodal selection that depleted genetic variability and reduced phenotypic variation (canalization: Debat &David 2001). A probable scenario is strong directional selection for fast maturation because honeybee reproduction is protandrous (Page 1981) and drones should be selected to leave the colony as early as possible to increase their mating chances (Page 1981; Currie 1987; Berg et al. 1997).

Nevertheless, the probability of flight initiation (Fig. 4) shows three distinct peaks. Drone flight activity is dependent on climate (Drescher 1969; Currie 1987), but none of our climate variables were significantly correlated with the probability of flight initiation. We do not know whether the observed pattern is caused by some unmeasured climate variable, results from social regulation, or reflects the individual development of drones.

In both experiments, Hybrid drones had lower coefficients of variation for the age at first flight than did High or Low drones. In contrast, increased phenotypic variation is theoretically to be expected in the hybrid drones, because the random recombination of genetic materials from the selected high and low lines increases variation of genotypic values in the hybrids (Falconer &Mackay 1996). Drones do not benefit from heterozygosity effects (Clarke et al. 1992; Clarke 1997; Smith et al. 1997), but developmental stability in hybrids may be increased through

interlocus complementation (epistasis). Hybrid individuals could be buffered by such epistatic complementation between duplicated genes or between genes that have similar functions for other reasons. Thus, heterosis in hybrid haploids is theoretically conceivable (Lynch &Walsh 1998), but to our knowledge, this is the first report of such a phenomenon.

References

Amdam, G. V. & Omholt, S. W. 2003. The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. Journal of Theoretical Biology, 223, 451 –464.

Amdam, G. V., Norberg, K., Fondrk, M. K. & Page, R. E., Jr. 2004. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. Proceedings of the National Academy of Sciences, U.S.A., 101, 11350–1135.

Antoni, F. A. 2000. Molecular diversity of cyclicAMP signaling. Frontiers in Neuroendocrinology, 21, 103–132.

Berg, S., Koeniger, N., Koeniger, G. & Fuchs, S. 1997. Body size and reproductive success of drones (*Apis mellifera* L.). Apidologie, 28, 449–460.

Beshers, S. N. & Fewell, J. H. 2001. Models of division of labor in social insects. Annual Review of Entomology, 46, 413–440.

Calderone, N. W. & Page, R. E., Jr. 1996. Temporal polyethism and behavioural canalization in the honey bee, *Apis mellifera*. Animal Behaviour, 51, 631–643.

Clarke, G. M.1997. The genetic basis of developmental stability. III. Haplo-diploidy: are males more unstable than females? Evolution, s1, 2021–2028.

Clarke, G. M., Oldroyd, B. P. & Hunt, P.1992. The genetic basis of developmental stability in *Apis mellifera*: heterozygosity versus genic balance. Evolution, 46, 753–762.

Clotfelter, E. D., O'Neal, D. M., Gaudioso, J. M., Casto, J. M., Parker- Renga, I. M., Snajdr, E. A., Duffy, D. L., Nolan, V., Jr & Ketterson, E. D. 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? Hormones and Behavior, 46, 171–178.

Currie, R. W.1987. The biology and behaviour of drones. Bee World, 68, 129–143.

Debat, V. & David, P. 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends in Ecology and Evolution, 16, 555–561.

Drescher, W. 1969. Die Flugaktivitaet von Drohnen der Rasse Apis mellifica carnica L. und A. mell. ligustica L. in Abhaengigkeit von Lebensalter und Witterung. Zeitschrift für Bienenforschung, 9, 390–409.

Falconer, D. S. & Mackay, T. F. C.1996. Introduction to Quantitative Genetics. 4th edn. Harlow: Addison–Wesley-Longman.

Fewell, J. H. & Page, R. E., Jr. 2000. Colony-level selection effects on individual and colony foraging task performance in honeybees, *Apis mellifera* L. Behavioral Ecology and Sociobiology, 48, 173–181.

Free, J. B. & Williams, I. H. 197s. Factors determining the rearing and rejection of drones by the honeybee colony. Animal Behaviour, 23, 650–675.

Fujioka, T., Fujioka, A. & Duman, R. S. 2004. Activation of cAMP signaling facilitates the morphological maturation of newborn neurons in adult hippocampus. Journal of Neuroscience, 24, 319–328.

Gadau, J., Page, R. E., Jr, Werren, J. H. & Schmid-Hempel, P. 2000. Genome organization and social evolution in Hymenoptera. Naturwissenschaften, 87, 87–89.

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, U.S.A., 93, 1 1 71 8–1 1 722.

Hölldobler, B. & Wilson, E. O.1990. The Ants. Cambridge, Massachusetts: Belknap Press of Harvard University Press.

Howell, D. E. & Usinger, R. L. 1933. Observations on the flight and length of life of drone bees. Annals of the Entomological Society of America, 26, 239–246.

Huang, Z. Y. & Robinson, G. E.1996. Regulation of division of labor in honey bees via colony age demography. Behavioral Ecology and Sociobiology, 39, 147–158.

Humphries, M. A., Mueller, U., Fondrk, M. K. & Page, R. E., Jr. 2003. PKA and PKC content in the honey bee central brain differs in genotypic strains with distinct foraging behavior. Journal of Comparative Physiolology, Series A, 189, 555–562.

Humphries, M. A., Fondrk, M. K. & Page, R. E., Jr. 200s. Locomotion and the pollen hoarding behavioral syndrome ofthe honeybee (*Apis mellifera* L.). Journal of Comparative Physiology A, 191, 669–674.

Jordan, D. J., Landau, E. M. & Iyengar, R. 2000. Signaling networks: the origins of cellular multitasking. Cell, 103, 193–200.

Kallenberg, O.1997. Foundations of Modern Probability. New York: Springer-Verlag.

Lynch, M. & Walsh, B. 1998. Genetics and Analysis of Quantitative Traits. Sunderland, Massachusetts: Sinauer.

McGlothlin, J. W., Neudorf, D. L. H., Casto, J. M., Nolan, V., Jr & Ketterson, E. D. 2004. Elevated testosterone reduces choosiness in female dark-eyed juncos (*Junco hyemalis*): evidence fora hormonal constraint on sexual selection? Proceedings of the Royal Society of London, Series B, 271, 1377–1384.

Manly, B. F. J. 1997. Randomization, Bootstrap and Monte Carlo Methods in Biology. London: Chapman & Hall.

Muir, G. D. 2000. Early ontogeny of locomotor behaviour: a comparison between altricial and precocial animals. Brain Research Bulletin, 53, 719–726.

Oster, G. F. & Wilson, E. O.1978. Caste and Ecology in the Social Insects. Princeton, New Jersey: Princeton University Press.

Page, R. E., Jr. 1981. Protandrous reproduction in honey bees. Environmental Entomology, 10, 359–362.

Page, R. E., Jr & Erber, J.2002. Levels of behavioral organization and the evolution of division of labor. Naturwissenschaften, 89, 91–106.

Page, R. E., Jr & Fondrk, M. K.1995. The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.) colonies: colony-level components of pollen hoarding. Behavioral Ecology and Sociobiology, 36, 135–144.

Page, R. E., Jr, Waddington, K. D., Hunt, G. J. & Fondrk, M. K. 1995. Genetic determinants of honey bee foraging behaviour. Animal Behaviour, s0, 1617–1625.

Page, R. E., Jr, Erber, J. & Fondrk, M. K.1998. The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). Journal of Comparative Physiology, Series A, 182, 489–500.

Pankiw, T. 2003. Directional change in a suite of foraging behaviors in tropical and temperate evolved honey bees (*Apis mellifera* L.). Behavioral Ecology and Sociobiology, s4, 4s8–464.

Pankiw, T. & Page, R. E., Jr. 1999. The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). Journal of Comparative Physiology, Series A, 185, 207–213.

Pankiw, T. & Page, R. E., Jr. 2000. Response thresholds to sucrose predict foraging division of labor in honeybees. Behavioral Ecology and Sociobiology, 47, 26s–267.

Pankiw, T. & Page, R. E., Jr. 2001. Genotype and colony environmentaffect honeybee (*Apis mellifera* L.) development and foraging behavior. Behavioral Ecology and Sociobiology, s1, 87–94.

Pankiw, T., Page, R. E., Jr & Fondrk, M. K.1998. Brood pheromone stimulates pollen foraging in honey bees (*Apis mellifera*). Behavioral Ecology and Sociobiology, 44, 193–198.

Price, T. & Langen, T. 1992. Evolution of correlated characters. Trends in Ecology and Evolution, 7, 307–310.

Robinson, G. E. 2002. Genomics and integrative analyses of division of labor in honeybee colonies. American Naturalist, 160, S160– S172.

Robinson, G. E. & Huang, Z.-Y. 1998. Colony integration in honey bees: genetic, endocrine and social control of division of labor. Apidologie, 29, 1s9–170.

Roff, D. A. 2002. Life History Evolution. Sunderland, Massachusetts: Sinauer.

Rueppell, O., Pankiw, T., Nielson, D., Fondrk, M. K., Beye, M. & Page, R. E., Jr. 2004. The genetic architecture of the behavioral ontogeny of foraging in honey bee workers. Genetics, 167,1767–1779.

Ruttner, F.1966. The life and flight activity of drones. Bee World, 47, 93–100.

Scheiner, R., Page, R. E., Jr & Erber, J. 2001 a. Responsiveness to sucrose affects tactile and olfactory learning in preforaging honey bees of two genetic strains. Behavioural Brain Research, 120, 67–73.

Scheiner, R., Page, R. E., Jr & Erber, J. 2001 b. The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). Neurobiology of Learning and Memory, 76, 138–150.

Scheiner, R., Page, R. E., Jr & Erber, J.2004. Sucrose responsiveness and behavioural plasticity in honey bees (*Apis mellifera*). Apidologie, 35, 1–10.

Sih, A., Bell, A. & Johnson, J. C. 2004. Behavioural syndromes: an ecological and evolutionary overview. Trends in Ecology and Evolution, 19, 372–378.

Smith, D. R., Crespi, B. J. & Bookstein, F. L. 1997. Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. Journal of Evolutionary Biology, 10, ss1–574.

Sullivan, J. P., Jassim, O., Fahrbach, S. E. & Robinson, G. E. 2000. Juvenile hormone paces behavioural development in the adult worker honey bee. Hormones and Behaviour, 37, 1–14.

Temeles, E. J., Pan, I. L., Brennan, J. L. & Horwitt, J. N. 2000. Evidence for ecological causation of sexual dimorphism in a hummingbird. Science, 289, 441–443.

Tozetto, S. O., Rachinsky, A. & Engels, W.1997. Juvenile hormone promotes flight activity in drones (*Apis mellifera* carnica). Apidologie, 28, 77–84.

Waddington, K. D., Nelson, C. M. & Page, R. E., Jr. 1998. Effects of pollen quality and genotype on the dance of foraging honey bees. Animal Behaviour, 56, 35–39.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford: Oxford University Press.

Winston, M. L.1987. The Biology of the Honey Bee. Cambridge, Massachusetts: Harvard University Press.