

Juvenile hormone and division of labor in honey bee colonies: effects of allatectomy on flight behavior and metabolism

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Accepted 4 April 2003

Summary

Three experiments were performed to determine why removal of the corpora allata (the glands that produce juvenile hormone) causes honey bees to fail to return to their hive upon initiating flight. In Experiment 1, the naturally occurring flights of allatectomized bees were tracked with radar to determine whether the deficit is physical or cognitive. The results indicated a physical impairment: allatectomized bees had a significantly slower ground speed than sham and untreated bees during orientation flights, but otherwise attributes such as flight range and area were normal. Flight impairment was confirmed in Experiment 2, based on observations of takeoff made in the field at the hive entrance. The allatectomized group had a significantly smaller percentage of flightworthy bees than did the sham and

untreated groups. Experiment 3 confirmed the flight impairment in laboratory tests and showed that allatectomy causes a decrease in metabolic rate. Allatectomized bees had significantly lower metabolic rates than untreated and sham bees, while allatectomized bees receiving hormone replacement had intermediate values. These results indicate that allatectomy causes flight impairment, probably partly due to effects on metabolic rate. They also suggest that juvenile hormone plays an additional, previously unknown, role in coordinating the physiological underpinning of division of labor in honey bee colonies.

Key words: *Apis mellifera*, behavioral development, corpora allata, division of labor, flight, honey bee, juvenile hormone, metabolism.

Introduction

Division of labor in honey bee colonies is based on a stereotyped pattern of behavioral development by adult workers (Winston, 1987). Young bees work in the hive for the first 2–3 weeks, performing such tasks as feeding brood (nursing) and hive maintenance. Young bees also take a series of orientation flights prior to the onset of foraging, to learn the location of the hive and prominent landmarks in the landscape, to facilitate navigation during foraging (Becker, 1958; Capaldi et al., 2000). Bees older than 2–3 weeks of age forage for nectar and pollen outside the hive for the remainder of their 4- to 6-week lives.

The regulation of division of labor by honey bees involves several physiological changes, including changes in the level of circulating juvenile hormone (JH) (Robinson, 2002). JH levels rise before the onset of foraging (Elekovich et al., 2001; Jassim et al., 2000) and typically are much higher in foragers compared with nurses (reviewed by Bloch et al., 2002). In addition, treatment with the JH analog methoprene results in precocious development of foraging (reviewed by Bloch et al.,

2002). Recently it was discovered that JH does not activate foraging, but rather is involved in controlling the pace at which bees develop into foragers (Sullivan et al., 2000). Bees that have had their corpora allata (the sole source of JH) surgically removed still became foragers, but at an older age than intact bees. Treatment with methoprene after allatectomy eliminated this delay (Sullivan et al., 2000).

Sullivan et al. (2000) also reported that allatectomy had another effect on honey bees: it caused them to disappear around the time they began taking their first orientation flight. This effect does not appear to reflect direct allatectomy-induced mortality, because there were no differences in mortality within the hive, but it was correlated with the onset of flight activity. Additional observations (Sullivan, 2001), conducted in different years on bees of different genotypes, in varied landscapes, confirmed that allatectomy can decrease the probability of returning to the hive upon initiating flight.

Sullivan et al. (2000) suggested that this allatectomy effect was due to a deficit in either the physical aspects of flight

behavior or the ability to learn/remember the location of the hive. Both suggestions are plausible. JH affects flight muscles (Rankin, 1989; Wyatt and Davey, 1996) and respiratory metabolism (Novak, 1966) in other insects. Experiments with a *Drosophila* cell line have shown that exposure to JH causes an increase in cytochrome oxidase activity and protein synthesis in the mitochondria (Stepien et al., 1988). In addition, there is a rich literature of hormone effects on cognition in vertebrates (reviewed by Bottjer and Johnson, 1997; McEwen, 2000; Pfaff et al., 2000; Welberg and Seckl, 2001), and a recent report of effects of JH on performance of honey bees in a learning assay (Maleszka and Helliwell, 2001).

Three experiments were performed to determine the nature of the allatectomy effect that causes honey bees to fail to return to their hive upon initiating flight. In Experiment 1, the naturally occurring flights of allatectomized bees were tracked using radar (Capaldi et al., 2000) to obtain indications about the nature of the deficit. We reasoned that flight tracks might reveal deficits in navigation expressed as errors in flight path, while a physiological deficit would be suspected if allatectomy caused effects on flight distance, duration or speed. In Experiment 2, intensive observations were made at the hive entrance to find evidence of allatectomy-induced flight impairment, especially during take-off.

Experiment 3 was performed in the laboratory, after the results from Experiments 1 and 2 indicated that allatectomized bees had impaired flight. We tested the hypothesis that allatectomy causes a decrease in metabolic rate; such a decrease might explain the flight impairment because of the intense energetic demands of flight. Just as there is an age-related increase in circulating JH, there are also age-related increases in both honey bee metabolic rate (Harrison, 1986) and the abundance of key respiratory metabolic enzymes in flight muscle (Maruyama and Moriwaki, 1958; Herold and Borei, 1963; Herold, 1965), with foragers showing the highest levels. In addition, forced flight in the laboratory complements the field analyses in Experiments 1 and 2 by providing a context that is independent of any motivation to fly or forage.

Materials and methods

Bees

Experiment 1 was conducted at the Rothamsted Institute for Arable and Crop Research, Harpenden, Hertfordshire, UK. Experiment 2 was conducted at the University of Illinois Bee Research Facility, Urbana, Illinois, USA. Experiment 3 was conducted at Arizona State University, Tempe, Arizona, USA. In all locations the bees were a mixture of European subspecies of the honey bee *Apis mellifera* L. All bee colonies were maintained according to standard beekeeping techniques. To obtain young adult bees for allatectomy, honeycomb frames containing pupae were removed from large colonies in the field and placed in an incubator (33°C, 95% relative humidity). Bees from different source colonies were used both for each experiment and for each replicate within an experiment. In some cases (noted below) the bees in a replicate were the

offspring of a queen instrumentally inseminated with semen from a single drone (due to haplodiploidy, their coefficient of relatedness was 0.75). In other cases the bees were the offspring of a queen that mated naturally (presumably with multiple males).

Allatectomy

Allatectomy was performed as described in Sullivan et al. (2000) on adult bees <6 h old (the cuticle is sufficiently elastic at this time to avoid cracking and bleeding following incision). Before treatment, bees were transferred into small cages placed in an incubator (33°C, 95% relative humidity) with access to honey in the comb and water *ad libitum*. Bees were immobilized prior to surgery by transfer to a small glass vial placed on crushed ice for approximately 3 min. Previous studies revealed that cold anesthesia has no effects on bee flight (Ebadi et al., 1980; Robinson and Visscher, 1984). After anesthetization, bees were mounted in Plasticine on the stage of an Olympus SZH10 stereo microscope and an incision was made across the back of the head. Each corpus allatum was grasped and removed with forceps; the cuticle then rapidly resumed its original shape, thereby sealing the incision. Sham allatectomy was identical except the CA were only gently touched with the tip of the forceps. Untreated bees were anesthetized, as were sham and allatectomized (CA-) bees, but were otherwise unmanipulated. Sullivan et al. (2000) validated this allatectomy technique with measurements of JH titer and histology and found no or extremely low levels of JH, and no evidence for regeneration of the CA.

After surgery, each CA- bee was marked for individual identification with a numbered, colored plastic tag (Opalithplättchen, Chr. Graze, KG, Endersbach, Germany) on the thorax and a spot of paint (Testor's PLA) on the dorsal tip of the abdomen. Bees were returned to the same cage in the incubator prior to introduction to an unrelated host colony. Bees were introduced by opening the cage and placing it over a hole in the top of the hive; treated bees entered the host colony overnight. Descriptions of host colonies are given for each experiment.

Hormone replacement was used in Experiment 3. CA- bees were treated by applying 200 µg methoprene/5 µl acetone to the dorsal abdomen when they were 2 days old. This dose is sufficient to eliminate the delay in age at onset of foraging caused by allatectomy (Sullivan et al., 2000), and is used routinely to induce precocious foraging (Bloch et al., 2002). The dose that is used is high, but methoprene, though more resistant to JH-specific degradative enzymes than native JH (Weirich and Wren, 1973), is also broken down rapidly. More than 69% of a methoprene treatment applied to ants (*Solenopsis invicta*) was broken down in 24 h (Bigley and Vinson, 1979). In addition, treatment with the same dose of an inactive JH analog has no behavioral effects, suggesting that methoprene's effects are due to hormonal activity (Robinson, 1987).

Hormone replacement was not employed in Experiments 1 and 2 because this treatment did not overcome the 'missing bee

effect' first noted in Sullivan et al. (2000), suggesting that a different timing, delivery or treatment may be required in this context. Bees in Experiment 3 were held in the cage in the incubator for an additional day prior to introduction to their host colony.

Experiment 1: Effects of allatectomy on radar-tracked flight behavior

Harmonic radar was used to track orientation flights of bees over flat farmland (700 m×900 m) at Rothamsted, UK using established methods (Riley et al., 1996; Capaldi et al., 2000). Harmonic radar detects the position signal of a transponder attached to the thorax of the bee by sending one wavelength and receiving a unique harmonic frequency re-radiated from the transponder every 3 s. The transponder consisted of a 16 mm vertical dipole aerial and a Schottky diode weighing 12 mg, less than the average mass of a load of nectar or pollen (Winston, 1987). No significant effects of the transponder on honey bee flight behavior have been detected (Capaldi et al., 2000).

A total of approximately 250 untreated, sham and CA– bees (derived from a naturally mated queen) were introduced to the host colony in cohorts of approximately 25 per treatment group over 11 days. The host colony occupied one British standard hive box and had an adult population of about 10 000 bees with a naturally mated queen. The hive entrance was observed from 08:30 h to 17:30 h daily, except during rain. An extended entrance board was attached to the hive; it had an acrylic cover and several sliding locks that separated incoming and outgoing bees, to facilitate capturing focal bees (leaving the hive naturally) and fitting them with a transponder (Capaldi et al., 2000). The departure behavior of transponder-affixed bees was observed by eye. Observers at the hive entrance were informed of flight progress by members of the research team monitoring the radar and recaptured the bee to remove the transponder upon return to the hive. Each bee was tracked once.

Tracking was conducted only when blue sky was visible. Anemometry stations in five locations took wind velocity readings at 9 s intervals to calculate the average wind vector for each track. Wind speed during tracking of CA–, sham and untreated bees did not vary significantly (analysis of variance, ANOVA: $F_{(2,82)}=2.61$, $P=0.08$). The colony was located in the center of plots of cereals and blooming oilseed rape and field beans 260 m NNW of the radar. Radar detection ranges in this landscape were 700 m radially and 3 m above the ground. Within a reference frame centered on the hive, bees were tracked 500 m to the west, 500 m to the east, 325 m to the south, and 325 m to the north. The limits of the flight area were detected by tracking long-distance foraging flights (results to be presented elsewhere); no orientation flight extended beyond the range of the radar.

The radar tracks were used to calculate the following details of orientation flights (Capaldi et al., 2000): flight duration (s), round trip distance (m), maximum range (m), area covered (m²) and mean ground speed (m s⁻¹). Duration was the total time the bee was tracked. Round trip distance was the sum of

the linear distances between one point in space occupied by a bee and its subsequent position, from the first to the last radar signal. A linear flight path was assumed between every two positions, regardless of the period of time elapsed between them. Maximum range was the distance of greatest radial departure from the hive. Area covered was calculated using the minimum convex polygon method of home range analysis from the Antelope spatial analysis software package (http://www.nbb.cornell.edu/neurobio/jbsv_downloads/programs.html). Ground speed was calculated by averaging the point-to-point speed of the moving bee based on 3 s radar sampling intervals. Track segments >9 s in duration were used for this calculation, to match flight-speed sampling intervals to wind-speed information. In cases when a radar track had a missing signal, the calculated flight speed ignored the time the bee spent in these gaps unless the plotted data indicated that the flight path continued on the same course and at the same speed. The proportion of time spent in gaps probably represents short landings on vegetation; it was not correlated with age or the number of flights taken by each bee (data not shown). Tracks of foragers were identified based on departure behavior, track shape (Capaldi et al., 2000), or return with pollen loads or a distended abdomen; these were excluded (1 CA–, 3 sham, 1 untreated).

Statistical analyses of radar data were conducted as follows. Normality was tested and log- or square-root transformation used when appropriate (Zar, 1996). ANOVA was performed to determine the effects of allatectomy on flight duration, round trip distance, maximum range, area covered and ground speed, followed by the least significant difference multiple-comparison test (SPSS 10.0.5 software, SPSS, Inc., Chicago, IL, USA). Outliers were excluded (2 CA–, 4 sham, 4 untreated) after testing the coefficient of symmetry (g_1) for highly significant ($P<0.001$) departure from normality (Grubbs, 1969; Zar, 1996). χ^2 analyses of the proportions of bees from each treatment group that failed to fly with the transponder or did not return from a flight (SAS 6.12 software) were followed by pairwise comparisons corrected for continuity.

Experiment 2: Effects of allatectomy on flight ability and survival

The behavior of CA– bees as they departed on orientation flights was studied by making detailed observations at the hive entrance. Three serial trials (replicates) of this experiment were performed with the same host colony, each time with approximately 50 untreated, 50 sham and 50 CA– bees derived from a (different) queen instrumentally inseminated with semen from a single drone. The host colony occupied one Langstroth hive box and had an adult population of about 12 000 bees and a naturally mated queen, with 6 frames of honeycomb containing brood and 4 frames of comb containing honey and pollen.

Observations were recorded on audio cassettes from 13:00 h to 18:00 h daily when the bees were 2–14 days of age. An extended entrance covered with Plexiglas (15 cm×15 cm) facilitated observation of identification tags on the bees. To

induce bees to walk with their tags facing upward, a thin film of petroleum jelly was applied on the underside of the Plexiglas at the end touching the hive (Winston and Katz, 1982). Flight ability was assessed based on criteria described below. In addition, we determined the age at first entrance appearance (based on the age at which a bee walked out of the hive into any part of the entrance) and the age at first flight.

The following procedure was used to obtain additional information on flight ability during times when no observations were taking place. The host colony was set on a stand inside two swimming pools (1.5 m and 3 m diameter, each containing at least 5 cm of water). We assumed that the pools would act as water traps and only bees with impaired flight ability would end up in them. Observations (Sullivan, 2001) confirmed this assumption. A circle of closely mown grass about 6 m in diameter surrounded the pools to further facilitate observation of marked bees on the ground. Mortality was monitored by installing a dead bee trap prior to the onset of observations (Gary and Lorenzen, 1984). In addition, censuses of the host colony were performed every other day by visually inspecting each frame of honeycomb in the hive twice and recording the identity of each bee present on audio tape. We positioned several small colonies near the host colony to help recover any marked bees that drifted (only 1 bee in all three replicates). Flight ability was categorized as: 'Flightworthy', 'Impaired Flight', 'Hive Bee', 'Unknown' and 'Dead'.

The 'Flightworthy' category was composed of bees that foraged or flew normally. Flightworthy foragers took multiple flights >15 min duration or returned from a flight with pollen or a distended abdomen (forager). Flightworthy pre-foragers showed normal flight behavior but were not observed to forage. The 'Impaired Flight' category was composed of missing fliers, weak fliers and flightless bees. Missing fliers took at least one successful (roundtrip) flight, but did not return from a subsequent flight. Some missing fliers probably occurred due to random factors such as predation rather than impairment. We included all missing fliers in the 'Impaired Flight' category; if this caused a bias it would be to decrease the magnitude of any observed treatment effects on flight. Weak fliers were those found in the pools. Observations of some of the bees that ended up in the water revealed that they climbed up the front of the hive, lifted the front legs and took off, but then flew down into the pool. These bees then 'swam' slowly and were unable to pull themselves from the water onto the hive stand. This was distinct from casual observations of bees foraging for water, which were able to get out of the pools. Flightless bees did not fly, and fell from the entrance after attempting to take off. Flightless bees had weak wing motion; the wings were not moving rapidly enough to even become blurred to the eye of the observer. The 'Hive Bee' category was composed of bees present in the hive at the end of the experiment and observed during censuses or at the entrance, but never observed to attempt to fly. The 'Unknown' fate category was composed of bees never observed or only observed in a census or at the entrance but not present in the hive at the end of the experiment; their corpses were not

recovered in either the dead bee trap or the pools. 'Dead' bees were corpses that were retrieved from the dead bee trap or from within the hive during a census.

Statistical analyses of the effects of allatectomy on flight ability were performed as follows. Contingency table analysis was used to compare the distributions of bees in the five categories. ANOVA was performed on age at first appearance at the entrance and age at first flight, after data were square-root-transformed to meet the assumptions of normality. Survival was calculated based on data from the observations, water traps, dead bee traps and censuses, adjusted for censorship due to termination of the experiment. Differences in survival between groups were analyzed by Kaplan–Meier survival analysis (yielding the Breslow statistic, *B*), performed with SPSS 10.0.5 software.

Experiment 3: Effects of allatectomy on metabolic rates

Bees for this experiment were taken from three small host colonies. Each host colony occupied a small hive and had an adult population of about 3000–4000 bees and a naturally mated queen, with 1 honeycomb frame containing honey and pollen and 1 containing brood. 2–3 cohorts of 1-day-old bees (derived from 2 naturally mated queens) were added to each host colony, about 25 bees per treatment group: CA–, CA–treated with methoprene (MCA–), sham and untreated. The hive entrance was closed until bees were sampled at about 8 days of age (see below) to ensure that they had had no flight experience prior to testing. Sampling was performed by removing a hive top and randomly collecting focal bees from the tops of the honeycomb frames and placing them in small glass vials. They were then transferred into a small cage and incubated (35°C) with access to honey and water *ad libitum* until assayed. Bees were collected when they were about 8–9 days old (untreated: 8.2 ± 0.01 , $N=102$; sham: 8.2 ± 0.01 , $N=92$; CA–: 8.0 ± 0.01 , $N=89$; and MCA–: 8.8 ± 0.02 , $N=58$; means \pm S.E.M.).

Flight ability was assessed in the laboratory (room temperature: 26°C) immediately before measuring metabolic rate. To assay flight ability, a bee was removed from the cage in the incubator and transferred to an open glass vial; bees uniformly attempted to initiate flight within 1 min. 'Flightless' bees fell immediately to the floor; 'Poor Fliers' descended gradually to the floor; and 'Flightworthy' bees flew up to a fluorescent light or window. Poor Fliers were retrieved and given a second opportunity to fly; in all cases the result was the same. All bees measured for metabolic rate were also tested for flight ability except for 13 untreated, 11 sham, 13 CA– and 13 MCA– individuals (due to logistical limitations).

We used whole body *in vivo* measurement of metabolic rate under forced flight conditions. This technique provides a good estimate of the metabolic capacity of honey bee flight muscles, given that flight muscles consume more than 90% of the O₂ taken up by the body when active (Rothe and Nachtigall, 1989; Suarez, 1992). Metabolic rate was assayed using a standard respirometry technique validated for the honey bee (Harrison, 1986; Harrison and Hall, 1993; Suarez et al., 1996) that

measures CO₂ production. O₂ consumption and CO₂ production are regarded as interchangeable estimates of metabolic rate under steady-state activity for honey bees; their metabolism of hexose sugars during flight is completely aerobic, yielding a respiratory exchange ratio of 1.0 for O₂ and CO₂ (e.g. Beenackers, 1969; Micheu et al., 2000). A bee was placed in a 20°C respirometry chamber (a 300 ml Plexiglas cylinder) and the chamber was flushed for 2 min at 2 l min⁻¹ with dry (Drierite, W. A. Hammond Drierite Co. Ltd., Xenia, OK), CO₂-free (Ascarite, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) air. Flight metabolic rate was then measured over 1 min of flight using flow-through respirometry (Harrison et al., 1996). The chamber was shaken and beaten with a padded stick to agitate the bee and cause it to fly continuously during the time of measurement. A magnesium perchlorate column was used to remove water vapor from the air leaving the respirometry chamber, and the excurrent carbon dioxide was measured with a Licor 5152 carbon dioxide analyzer (± 1 p.p.m. CO₂, Lincoln, NE, USA). The flow rate of air through the chamber was approximately 0.8 l min⁻¹, measured to ± 0.001 l min⁻¹ with an Omega mass flow meter (Stamford, CT, USA). The rate of CO₂ production was measured once per second, digitized, and recorded with a Sable Systems AD-1 data acquisition system (Las Vegas, NV, USA).

Any bee that did not fly continuously throughout the trial was excluded. After respirometry, bees were removed from the chamber, cooled on ice, and weighed (to ± 0.1 mg; Mettler Toledo, Switzerland). CO₂ production rates were converted to metabolic rates assuming that only carbohydrates are catabolized during flight (Rothe and Nachtigall, 1989). Metabolic rate was represented as W g⁻¹ body mass.

Statistical analyses of the effects of allatectomy on flight ability and metabolic rate were performed as follows. Differences between the treatment groups in the proportion of individuals in each flight ability category were determined by contingency table analysis. Because the bees were different ages and derived from different queens, the Cochran–Mantel–Haenszel test was used to control for variation in age and genotype. Differences between treatment groups in metabolic rate were analyzed by ANOVA (on

square-root-transformed data), again controlling for variation in age and genotype, which are important variables (e.g., Allen, 1958; Harrison et al., 1996). SAS (SAS Institute Inc., Cary, NC, USA) and SPSS (SPSS, Inc., Chicago, IL, USA) software were used for statistical analyses. A correlation analysis was also performed, based on the *a priori* hypothesis that hormone replacement (*via* methoprene) would partially rescue an allatectomy deficit.

Results

Experiment 1: Effects of allatectomy on radar-tracked flight behavior

The percentage of bees unable to fly with the transponder differed significantly among the groups, with CA– bees showing the highest percentage [$\chi^2=12.45$, $P<0.01$; untreated, 9% (4/46); sham, 27% (22/83); CA–, 37% (37/101)]. Of those that could fly, CA– bees had a significantly slower ground speed than sham and untreated bees during orientation flights (Table 1). There were no other significant differences in flight attributes (Table 1), and there was no significant difference between the treatment groups in the percentage of bees that took an orientation flight and did not return [$\chi^2=0.20$, $P=0.91$; untreated, 4% (2/46); sham, 4% (3/83); CA–, 5% (5/101)].

Experiment 2: Effects of allatectomy on flight ability and survival

Survival varied significantly among the groups (Replicate 1: $B=35.32$, $P<0.00001$; Replicate 2: $B=23.36$, $P<0.00001$; Replicate 3: $B=28.15$, $P<0.00001$). The CA– and sham groups had significantly lower survival rates than the untreated group in all replicates (Fig. 1). The CA– group had significantly lower survival than the sham group in the first replicate but did not differ in the other replicates.

Decreased survival of CA– bees was reflected in lower proportions of Flightworthy CA– bees (Table 2). The distribution of bees classified as Flightworthy, Impaired Flight, Hive Bee, Unknown or Dead differed significantly between the treatment groups [$Q_{MH}(2)=57.86$, $P<0.001$]. The CA– group had the smallest percentage of Flightworthy bees, followed by

Table 1. Effects of allatectomy (CA–) on radar-tracked honey bee orientation flights

	Untreated (N=24)	Sham (N=25)	CA– (N=37)
Duration (s)	138 \pm 3 ^a	178 \pm 4 ^a	176 \pm 2 ^a
Round trip distance (m)	188 \pm 4 ^a	178 \pm 4 ^a	191 \pm 3 ^a
Maximum range (m)	73 \pm 2 ^a	63 \pm 1 ^a	65 \pm 1 ^a
Area covered (m ²)	1273 \pm 53 ^a	1094 \pm 54 ^a	1190 \pm 32 ^a
Mean ground speed (m s ⁻¹)	3.14 \pm 0.04 ^a	2.93 \pm 0.03 ^a	2.62 \pm 0.02 ^{b,1}

Values are means \pm S.E.M.

Means followed by the same letter across a row are not significantly different ($P>0.05$).

Mixed ANOVA was performed using wind speed as a covariate and age as a random factor for analyses of duration ($F_{(2,49)}=0.94$; $P=0.40$); round trip distance ($F_{(2,49)}=0.86$; $P=0.43$); maximum range ($F_{(2,49)}=1.32$; $P=0.28$); area covered ($F_{(2,49)}=1.09$; $P=0.34$); mean ground speed ($F_{(2,48)}=6.40$; $P<0.003$).

¹N=36.

For details of treatments, see text.

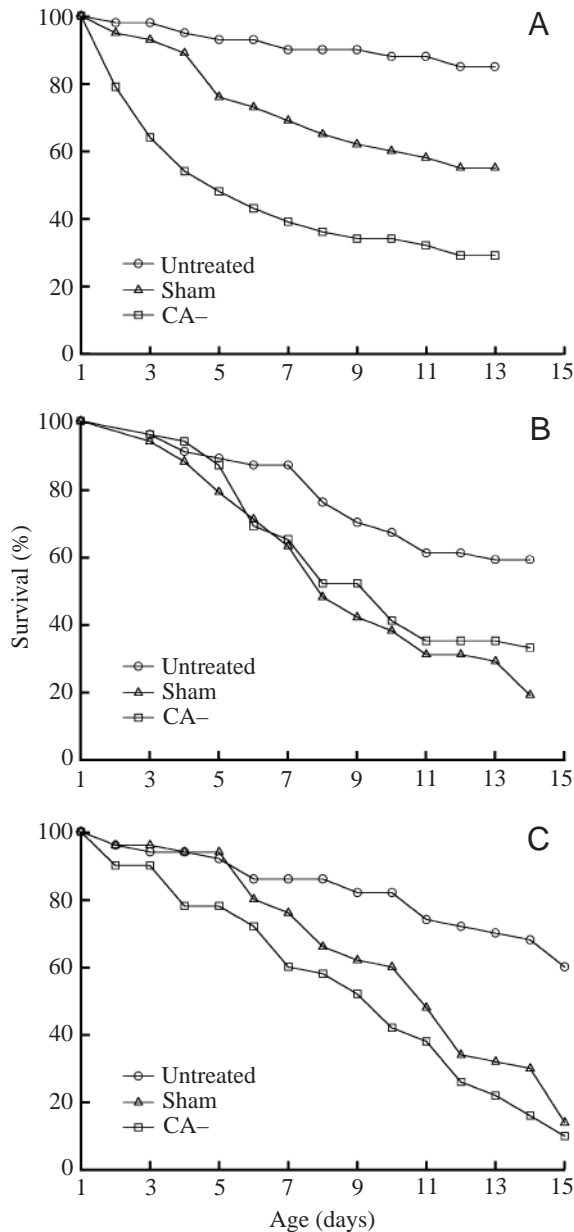


Fig. 1. Effects of allatectomy on honey bee survival. Survival data from three replicates (A–C) each of approximately 50 untreated, 50 sham and 50 CA– bees, were obtained from: daily hive entrance observations when the bees were 2–14 days of age, large water traps surrounding the hive to collect poor fliers, dead bee traps and censuses of the hive interior. Treatment groups followed by different letters showed significantly different survival (Kaplan–Meier survival analysis, $P < 0.00001$, see text).

the sham group and the untreated group. The CA– group had the largest percentages of Impaired Flight and Unknown bees.

In addition to flight impairment, bees from the CA– and sham groups appeared at the entrance and initiated flight at significantly younger ages than untreated bees (Table 3). There were also significant differences in the age at onset of flight for Impaired flight and Flightworthy bees in the untreated and

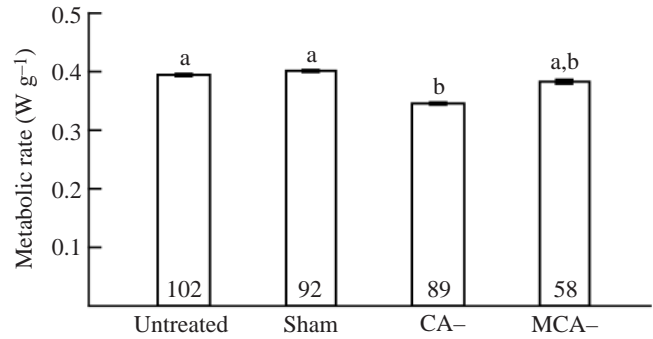


Fig. 2. Effects of allatectomy and hormone replacement on flight metabolic rate in honey bees. Bars with different letters had significantly different metabolic rates. Mixed ANOVA with age and queen source as random factors [$F_{(5,357)} = 2.62$; $P < 0.024$], followed by comparison of least-square means among the groups ($P < 0.05$). MCA–, allatectomized followed by treatment with the JH analog methoprene.

sham group, but not in the CA– group (Table 3). Untreated bees with impaired flight attempted to fly at a significantly younger age than untreated bees that flew successfully. The same pattern was observed in the sham group. In contrast, there was no significant difference in the CA– group between Impaired Flight and Flightworthy bees.

Experiment 3: Effects of allatectomy on metabolic rates

Flight ability in the laboratory also differed significantly among the groups [$Q_{MH}(10) = 26.39$, $P < 0.003$] (results not shown). The CA– group had the smallest percentage of Flightworthy bees (64%, $N = 76$), significantly less than for untreated and sham groups (88%, $N = 89$ and 83%, $N = 81$, respectively). CA– bees treated with methoprene (MCA–) were intermediate (69%, $N = 45$) and did not differ significantly from either the CA– group or the sham and untreated groups. Although relatively small, the percentage of CA– bees that were ‘Flightworthy’ in this experiment was higher than in Experiment 2. This might reflect differences between the laboratory and field; some bees in the CA– group that flew well in a warm, windless room may not have been able to do so under less favorable conditions in the field.

There were significant differences between the treatment groups in metabolic rate (Fig. 2). Bees in the CA– group had significantly lower metabolic rates than the untreated and sham groups. The MCA– group was ‘intermediate’ and did not differ significantly from either the CA– or the sham and untreated groups. To analyze the effects of hormone replacement further, a non-parametric correlation analysis was performed in which the four treatment groups were rank-ordered based on endocrine status and the prediction that methoprene treatment provides a partial rescue of the allatectomy effect on metabolic rate: CA– (1), MCA– (2), sham (3), untreated (4). There was a significant correlation between metabolic rate and endocrine status (Mantel–Haenszel Statistic = 6.813, $P < 0.01$).

Allatectomy-induced flight impairment appears to be a consequence of allatectomy-induced effects on metabolic rate.

Table 2. Effects of allatectomy (CA-) on honey bee flight ability and survival

Replicate	Treatment	Bee classification (%)				
		Flightworthy	Impaired flight	Hive bee	Unknown	Dead
1	Untreated (N=40)	65 (26)	13 (5)	23 (9)	0 (0)	0 (0)
	Sham (N=55)	51 (28)	26 (14)	16 (9)	7 (4)	0 (0)
	CA- (N=56)	18 (10)	54 (30)	14 (8)	11 (6)	4 (2)
2	Untreated (N=46)	65 (30)	15 (7)	4 (2)	13 (6)	2 (1)
	Sham (N=48)	56 (27)	27 (13)	2 (1)	15 (7)	0 (0)
	CA- (N=54)	22 (12)	48 (26)	4 (2)	24 (13)	2 (1)
3	Untreated (N=50)	62 (31)	18 (9)	10 (5)	2 (1)	8 (4)
	Sham (N=50)	42 (21)	46 (23)	0 (0)	8 (4)	4 (2)
	CA- (N=50)	36 (18)	34 (17)	0 (0)	20 (10)	10 (5)

Percentages may not sum to 100 due to rounding.

Actual numbers are given in parentheses.

For details of bee classification, see text.

For statistical analyses, see text.

Table 3. Effects of allatectomy (CA-) on age at first entrance appearance and flight initiation in honey bees

	First entrance appearance	First flight		
		All bees	Impaired flight	Flightworthy
Untreated	7.20±0.23 (113) ^a	7.77±0.27 (103) ^a	6.38±0.45 (16)	7.99±0.30 (87)*
Sham	6.08±0.18 (132) ^b	6.67±0.24 (108) ^b	5.59±0.41 (29)	7.06±0.29 (79)**
CA-	5.94±0.21 (117) ^b	6.60±0.27 (89) ^b	6.27±0.39 (49)	7.00±0.35 (40)

Values are mean age in days ±S.E.M. (N).

For the first and second columns, values within a column followed by the same letter are not significantly different ($P>0.05$); ANOVA was performed on square-root-transformed data for age at first entrance appearance ($F_{(2,353)}=10.92$; $P<0.0001$) and age at first flight ($F_{(2,291)}=7.51$; $P<0.001$).

For the third and fourth columns, asterisks indicate significant differences in age at first flight between 'Impaired flight' and 'Flightworthy' bees: * $P<0.05$, ** $P<0.01$; two-tailed t -tests.

For details of flight categories, see text.

The metabolic rate for Flightworthy bees was about twice as large as for Flightless bees with Poor Fliers intermediate; all flight categories were significantly different from each other (Table 4). This result was obtained regardless of treatment. There were significant differences in metabolic rates for bees in different flight categories for all bees pooled and for bees within each treatment group (Table 4).

Discussion

The results of these three independent studies contribute to our understanding of the regulation of flight by the corpora allata, one of the key endocrine organs in insects. They also suggest that JH is playing an additional, previously unknown, role in coordinating the physiological underpinnings of division of labor in honey bee colonies: influencing metabolic rate and flight ability.

Sullivan et al. (2000) reported that allatectomy caused bees to disappear around the time they began taking their first orientation flight. Our results confirm this effect and attribute it to allatectomy-induced flight impairment. Radar tracking and detailed observations at the hive entrance indicate that the

impairment does not involve cognitive deficits in navigation that result in bees becoming lost. Rather, allatectomized bees are physically impaired: many cannot achieve proper lift-off and those that can fly show reduced flight speeds. Hormone replacement was not employed in Experiments 1 and 2, so it is not possible to ascribe these effects of allatectomy conclusively to an absence of JH. In Experiment 3, hormone replacement partially eliminated the effects of allatectomy, though the effect was not overwhelming and was detected with one statistical analysis, but not another. These results provide some support for the interpretation that a JH deficiency due to allatectomy caused the effects on flight and metabolic rate reported here. The same type of methoprene treatment did not overcome the missing bee effect first noted by Sullivan et al. (2000), suggesting that a different timing, delivery or treatment may be required for a more robust rescue. Alternatively, allatectomy may have inadvertently affected other unknown processes that caused these effects; if so, they are unlikely to involve a blood-borne factor, as the honey bee corpora allata *in vitro* produce only one primary product, JH III (Huang et al., 1991).

Our results suggest that flight impairment in allatectomized

Table 4. *Effects of allatectomy (CA-) on metabolic rate, grouped by flight category*

	Flightless	Poor flier	Flightworthy	N
Untreated	0.25±0.02 (9) ^a	0.31±0.02 (2) ^b	0.41±0.00 (16) ^c	89
Sham	0.19±0.02 (6) ^a	0.33±0.01 (8) ^b	0.43±0.00 (67) ^c	81
CA-	0.22±0.01 (19) ^a	0.32±0.01 (8) ^b	0.39±0.00 (49) ^c	76
MCA-	0.13±0.01 (8) ^a	0.28±0.03 (6) ^b	0.45±0.00 (31) ^c	45
Pooled	0.20±0.00 (42) ^a	0.31±0.00 (24) ^b	0.42±0.00 (225) ^c	291

Values are mean metabolic rate ($W\ g^{-1}$) ±S.E.M. (N).

Across each row, values followed by a different letter are significantly different ($P < 0.05$; mixed ANOVA, performed with age and queen source as random factors followed by comparison of least-square means). Untreated: $F_{(2,84)}=6.79$; $P < 0.01$; Sham: $F_{(2,76)}=17.82$; $P < 0.0001$; CA-: $F_{(2,71)}=18.30$, $P < 0.0001$; MCA-: $F_{(2,40)}=26.85$; $P < 0.0001$; Pooled: $F_{(2,279)}=61.52$; $P < 0.0001$.

Within each flight category, there were no significant differences in metabolic rate (Flightless: $F_{(3,37)}=0.48$; $P=0.70$; Poor fliers: $F_{(3,18)}=1.95$; $P=0.16$; Flightworthy: $F_{(3,219)}=2.39$, $P=0.07$).

For details of flight categories, see text.

bees is a consequence of decreased metabolic rates, reflecting inadequate development and/or function of the flight muscles. Previous studies of the Colorado potato beetle *Leptinotarsa decemlineata* and the migratory locust *Locusta migratoria* implicated JH in the development and maintenance of flight muscles (Rankin, 1989; Wyatt and Davey, 1996), and JH treatment has been shown to affect flight ability in several insect species (reviewed by Rankin, 1989; Nijhout, 1994). But if allatectomy causes an impairment of flight in honey bees because of the absence of JH, why are all allatectomized bees not more equally affected (Sullivan et al., 2000; this study)? One possible explanation unexplored in this study is genetic variation, which has been shown to affect metabolic rate, muscle development, JH levels and rate of behavioral maturation (Coelho and Mitton, 1988; Nachtigall et al., 1995; Harrison et al., 1996; Giray et al., 2000). Also, if flight in allatectomized bees is impaired, why are the radar-tracked flights of allatectomized bees normal except for decreased ground speed? We speculate that the radar tracks of allatectomized bees show such a specific, limited deficit because only the least impaired bees of this group were capable of flying with the transponder. It should be noted that an additional possible influence on flight speed is experience. A previous radar study (Capaldi et al., 2000) showed that bees with more flight experience fly faster than less experienced bees. However, because the allatectomized and control bees used for this study were selected randomly with respect to flight experience and covered a range of ages (3–13 days), the most likely explanation is that allatectomy exerted a direct influence on flight speed.

Allatectomized bees were observed at the hive entrance and initiated flight at significantly younger ages than untreated bees. This appears to be a non-specific effect of surgery, because sham-operated bees showed the same response. There appears to be an intriguing interaction between flight impairment and the age at onset of flight. All bees – even those in the untreated group – that showed impaired flight also initiated flight at younger ages than did the Flightworthy bees. Failed flight may occur because some bees attempt to fly before the flight machinery has matured sufficiently to support this.

Perhaps the stress of surgery causes neurochemical and endocrine changes that cause an earlier onset of flight, effects that appear to be opposite to the delay in the onset of foraging caused by allatectomy (Sullivan et al., 2000). Starvation causes premature honey bee foraging (Schulz et al., 1998), but effects on pre-foraging orientation flights have not been studied. Starvation also causes an increase in octopamine immunoreactivity in the bee brain (Kaatz et al., 1994) and octopamine has been implicated in the stress response of several insect species (Nijhout, 1994). Octopamine is involved in the regulation of the onset of honey bee foraging (Schulz and Robinson, 2001), but not orientation (Schulz et al., 2002). It also is not clear why about 15% of the untreated bees showed impaired flight. These considerations suggest that the neural mechanisms that control the initiation of flight may operate somewhat independently of the physiological mechanisms that control flight ability in honey bees.

Bees in the sham group in Experiment 2 showed a deficit in flight ability but in Experiment 3 they did not. This might be because Experiment 2 involved younger bees than those in Experiment 3. If so, these results would be consistent with the sham effect involving a premature initiation of flight, as discussed above.

The metabolic rates reported here are comparable to those reported previously (reviewed by Harrison and Fewell, 2002) especially to those in Harrison (1986), which were also derived from relatively young bees, as in this study. Foragers have higher metabolic rates (Harrison and Fewell, 2002). Only allatectomized bees had values that were appreciably lower than those reported by Harrison (1986). Allatectomized bees treated with methoprene showed metabolic rates comparable to untreated bees in this study and that of Harrison (1986).

JH influences several aspects of adult honey bee maturation that are involved in division of labor, including exocrine gland secretions, responsiveness to olfactory task-related stimuli, age at onset of foraging (reviewed by Bloch et al., 2002), and levels of octopamine in the antennal lobes (Schulz et al., 2002). The results presented here suggest an additional role for JH in honey bees: maturation of flight ability *via* effects on metabolic rate. Given that removal of the corpora allata delays but does

not completely repress the onset of foraging (Sullivan et al., 2000), perhaps the effects of allatectomy reported here for young bees also reflect developmental delays. JH appears to coordinate the timing of various physiological and behavioral processes associated with honey bee maturation, thus improving the effectiveness of division of labor.

We thank A. E. Edwards, J. L. Osborne, J. R. Riley, D. R. Reynolds and A. D. Smith for operating the radar equipment and collaborating on the radar study; N. Carreck, K. Larsen, A. Martin and L. Pyter for assistance with the radar study; A. J. Ross, A. Toth and T. Tran for assistance with the flight departure study; and F. Delcomyn and J. Juraska for reading earlier versions of this manuscript as members of J.P.S.'s doctoral dissertation committee. Supported by NSF grants to S.E.F. and G.E.R. (IBN 9807560) and J.H.F. and J.F.H. (IBN 0093410) and a Travel Award from the Company of Biologists Limited to E.A.C.

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