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COLONY DEFENCE AND MALE BEHAVIOUR IN HONEYBEES *Apis mellifera* L.

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SUMMARY

Field tests on whole colonies and laboratory tests on individual worker honeybees were made to determine their defence behaviour. Individually marked 1-day old yellow (gentle genotype) and black (defensive genotype) drones were introduced into 3 gentle and 3 defensive colonies. The subsequent movement of drones away from home colonies and into host colonies (termed 'drifting') was recorded. Drones drifted away from defensive home colonies earlier than from gentle home colonies. Black drones drifted earlier than yellow drones. But both drone genotypes preferentially drifted into gentle colonies. This reproductive parasitism would theoretically lead to a spread of defensive behaviour in the population of honeybee colonies under investigation.

KEY WORDS
honeybee, defensive behaviour, laboratory tests, drone drifting

RESUME

La défence de la colonie et la conduite des drônes en l'abeille (Apis mellifera **L.)**

La conduite défensive étiat testé avec des colonies-complètes et, dans le laboratoire, avec des abeilles-individuelles. Drônes jaunes (génotype gentile) et drônes noirs (génotype défensive) étant marqué et introdué dans 3 colonies gentiles et 3 colonies défensives et leurs movements des «colonies-de-maison» et aux «colonies-reçues» (donné le nom «départ») étant mesurés. Drônes dans une colonie-de-maison défensive et drônes noirs départent quand ils sont plus jeunes que drônes dans une colonie-de-maison gentile. Drônes jaunes et noirs entrent dans les colonies gentles plus souvant que dans les colonies défensives. La population des colonies d'abeilles deviendra plus défensive à cause de la parasitisme des colonies gentiles par drônes.

MOTS CLÉS
l'abeille, conduite défensive, tests du laboratoire, movement des drônes

INTRODUCTION

Large variation exisits between honeybee colonies in their defence behaviour within one locality, not only in South and Central America where highly defensive Africanized honeybees (AHBs) can represent a public nuisance (MICHENER, 1975), but also in Europe. From an economic point of veiw, beekeepers waste much time when manipulating highly defensive colonies. The defence behaviour of a colony of honeybees is dependent upon a number of environmental and biotic variables such as ambient temperature and colony size. But there is also a large genetic component to honeybee colony defence (COLLINS, 1988).
Honeybees reproduce via swarms (and therefore new queens) and drones (males). Hence the drones with which a queen mates are just as important as the queen in determining a colony's defence behaviour. Given the mating system of honeybees at congregation areas (CURRIE, 1987), drones can act as highly dispersive spreaders of defensive genes.
RINDERER *et al.* (1985) have recently shown that drones from highly defensive AHB colonies in Venezuela preferentially move (drift) from their home colony to neighbouring gentle European honeybee (EHB) colonies, thereby reducing the host colony's drone production and theoretically leading to an over-representation of AHB drones (carrying defensive genes) at mating congregations. This effect is thought to have contributed to the establishment of AHBs in South and Central America (RINDERER *et al.*, 1988).
AHBs and EHBs differ in a number of important traits other than their defence behaviour. Hence a study was undertaken of drone drifting between defensive EHB colonies and gentle EHB colonies to determine whether defence *per se* was of importance in the differential drifting of drones.

MATERIALS AND METHODS

All experiments were performed at the UWCC Cleppa Park apiary, Cardiff. In 1987, a breeding regime following LAIDLAW and PAGE (1986) incorporating single-drone instrumental insemination of mother queens followed by open-mating of daughter queens was used to produce 2 genetic types of colonies, one gentle and one defensive. Three colonies of each type, headed by full-sister queens within each type, were arranged in a circle with alternating gentle and defensive colonies. Hive entrances faced inwards and colonies were separated by 1 meter from neighbouring colonies in the circle.
The defence behaviour of each colony was measured on 3 occasions at 1 month intervals, commencing May 1988, using methods of STORT (1974). The front of a hive was given a standard knock whilst a 4 cm diameter black suede ball suspended on a 10 cm length of string was jerked up and down 5 cm in front of the hive entrance. Sequential measurements were taken of: (i) the time from hive knocking to first stinging of the suede ball; (ii) the number of stings embedded in the suede ball in 1 minute following the first sting; and (iii) the distance the bees followed the suede ball when it was removed from the hive entrance after 1 minute of stinging. A laboratory test of one component of the defence behaviour of individual bees was also performed on 1-day old worker bees from 5 of the 6 colonies (see KOLMES and FERGUSSON-KOLMES, 1989a, 1989b for experimental details). Twenty bees (emerging from a comb of pupae placed overnight in a 34°C incubator) were individually housed over a grid of metal wires. An increasing voltage was applied to the wires until the first stinging response was seen.
Combs containing drone pupae were collected from each of the 6 experimental colonies on the 14 May 1988 and placed overnight in a 34°C incubator. On the following day, emerging adult drones were individually marked with numbered and coloured opalith disks. Drones from gentle colonies were pale in colour and are henceforth termed 'yellow drones'. Drones from defensive colonies were dark in colour and are henceforth termed 'black drones'. Forty yellow drones and 40 black drones were introduced

into each of the 6 experimental colonies immediately after being marked. These colonies represented the 'home' colonies.

The movement of drones between colonies (drifting) was measured by examining each hive in the morning, before drones commenced flight activity, and recording the identity of all marked drones found within each hive. These in-hive inventories were taken at weekly intervals following drone introduction. In addition, hive entrance observations were made on all days suitable for drone flight, 5, 7, 11, 16, 18, 20, 22, 28, 29 and 32 days following drone introduction. On such days, the observer moved in a clockwise direction around the experimental hives and recorded the identity of all marked drones entering and leaving one hive in a 3 minute period.

RESULTS

Defence behaviour

The defensive colony types were markedly and consistently more defensive than the gentle colony types for all whole-colony measures of defence behaviour (*table I*). In addition, individual worker bees from defensive colonies had a significantly lower threshold voltage to elicit stinging than individual worker bees from gentle colonies (*table I*), suggesting that individual worker bees from defensive colonies are more willing to sting relative to bees from gentle colonies. There is a close agreement between whole-colony behaviour and individual-bee responses.

	GENTLE COLONIES		DEFENSIVE COLONIES	
	MEAN	s.e.m.	MEAN	s.e.m.
TIME TO FIRST STING (seconds)	98.11	6.83	3.11	0.29
NUMBER OF STINGS IN 1 MINUTE	1.55	0.40	50.44	2.73
DISTANCE FOLLOWED (meters)	0	0	20.33	0.67
THRESHOLD VOLTAGE TO ELICIT STINGING (volts)	6.66	0.44	5.01	0.12

TABLE I. The responses of the 3 gentle colonies and 3 defensive colonies to measures of their defence behaviour, recorded on 3 separate occasions. Threshold voltages to elicit stinging were obtained from the laboratory testing of individual bees. In all cases, defensive colonies are significantly more defensive than gentle colonies (all comparisons of means by Mann-Whitney U tests, $p<0.05$).

Drone drifting in relation to colony type

In total, 58% of all marked drones drifted away from the home colony to which they were originally introduced. At 2 weeks post-introduction, more drones originally hived in defensive colonies drifted away from their home colony, relative to those drones hived in gentle colonies (*table II*). The reverse occurred at 3 weeks post-introduction (*table II*). The differential drifting of drones away from defensive home colonies at a young drone age may be a result of faster rates of drone maturation in defensive relative to gentle colonies.

But at both 2 and 3 weeks of age, drifting drones preferentially entered gentle colonies over defensive colonies (*table III*). At other drone ages, there was no differential movement into either colony type.

	COLONY IN WHICH DRONES WERE FIRST HIVED		χ^2
	GENTLE COLONY	DEFENSIVE COLONY	
WEEK 1			
NON-DRIFTING DRONES	151	101	0.25 *n.s.*
DRIFTING DRONES	8	7	
WEEK 2			
NON-DRIFTING DRONES	71	37	13.09 ***
DRIFTING DRONES	44	63	
WEEK 3			
NON-DRIFTING DRONES	41	48	6.79 **
DRIFTING DRONES	51	26	
WEEK 4			
NON-DRIFTING DRONES	25	24	0.26 *n.s.*
DRIFTING DRONES	31	36	
WEEK 5			
NON-DRIFTING DRONES	10	8	1.35 *n.s.*
DRIFTING DRONES	9	15	

TABLE II. The number of marked drones either remaining in their home colony or drifting away from their home colony are presented with respect to the defence behaviour of their home colony. Contingency table analyses compare the relative numbers of drifting drones from gentle and defensive colonies. Analyses are performed separately for each week as there is non-independence of data between weeks. ** $p<0.01$; *** $p<0.001$. Degrees of freedom = 1 for all analyses.

	COLONY WHICH RECEIVES DRIFTING DRONES		χ^2
	GENTLE COLONY	DEFENSIVE COLONY	
WEEK 1			
DRIFTING DRONES	8	7	0.10 *n.s.*
(EXPECTED NUMBER)	(7.4)	(7.6)	
WEEK 2			
DRIFTING DRONES	71	36	9.11 **
(EXPECTED NUMBER)	(55.4)	(51.6)	
WEEK 3			
DRIFTING DRONES	50	27	10.22 **
(EXPECTED NUMBER)	(36.0)	(41.0)	
WEEK 4			
DRIFTING DRONES	36	31	0.24 *n.s*
(EXPECTED NUMBER)	(34.0)	(33.0)	
WEEK 5			
DRIFTING DRONES	16	8	1.93 *n.s.*
(EXPECTED NUMBER)	(12.6)	(11.4)	

TABLE III. The number of marked drones that drift away from their home colony are presented with respect to the defence behaviour of the colony to which they drift. Observed numbers of drifting drones are compared with expected values that are derived from the numbers of drones departing from gentle and defensive colonies. Analyses are performed separately for each week as there is non-independence of data between weeks. ** $p<0.01$. Degrees of freedom = 1 for all analyses.

Drone drifting in relation to drone genotype

After both 1 and 2 weeks of life, black drones (the sons of queens heading defensive colonies) drift away from the colony in which they were originally introduced to a far greater extent than yellow drones (*table IV*). Subsequently, both black and yellow drones drift to an equal extent away from their home colonies (*table IV*), though there are no differences between drone types with respect to the colony to which drones drift (*table V*); both black and yellow drones tend to drift towards gentle colonies (*table III*).

Hive entrance observations provided information on only 1/6 th of the flight activity of marked drones. However, individual drones were seen to visit up to 4 different colonies on any one day and to do so over several days; drones are clearly very labile with respect to their movement. The mean age at first flight, as determined by hive-entrance observations, was similar for black and yellow drones (mean age ± s.e.m: black drones 14.72 ± 2.54 days; yellow drones 14.50 ± 2.28 days; difference between means t_{211} = 0.29, *n.s.*), suggesting that black and yellow drones mature at a similar rate.

	DRONE TYPE		χ^2
	YELLOW DRONES	BLACK DRONES	
WEEK 1			
NON-DRIFTING DRONES	135	117	9.17 **
DRIFTING DRONES	2	13	
WEEK 2			
NON-DRIFTING DRONES	62	46	4.47 *
DRIFTING DRONES	46	61	
WEEK 3			
NON-DRIFTING DRONES	50	39	1.90 *n.s.*
DRIFTING DRONES	35	42	
WEEK 4			
NON-DRIFTING DRONES	27	22	0.39 *n.s.*
DRIFTING DRONES	33	34	
WEEK 5			
NON-DRIFTING DRONES	9	9	1.19 *n.s.*
DRIFTING DRONES	16	8	

TABLE IV. The number of marked drones either remaining in their home colony or drifting away from their home colony are presented with respect to drone genotype. Contingency table analyses compare the relative numbers of drifting drones of each genotype. Analyses are performed separately for each week as there is non-independence of data between weeks. * $p<0.05$; ** $p<0.01$. Degrees of freedom = 1 for all analyses.

DISCUSSION

The defence behaviour of a colony of honeybees is difficult to quantify objectively, partly because it is the outcome of several variable responses by many individuals and partly because it is conditional upon many uncontrolled environmental variables. The threshold voltage laboratory test of individual worker bees (KOLMES and FERGUSSON-KOLMES, 1989a, 1989b), as used here, provides a more objective experimental paradigm for determining an aspect of the defence response of bees, the results of which are in general agreement with field tests of defence behaviour. This laboratory test is being used to evaluate the genetic basis of defence behaviour.

RECEIVING COLONY TYPE	DRIFTING DRONE TYPE: YELLOW DRONES	BLACK DRONES	χ^2
WEEK 1			
GENTLE COLONY	1	7	0.74 *n.s.*
DEFENSIVE COLONY	1	6	
WEEK 2			
GENTLE COLONY	32	39	0.37 *n.s.*
DEFENSIVE COLONY	14	22	
WEEK 3			
GENTLE COLONY	23	27	0.02 *n.s*
DEFENSIVE COLONY	12	15	
WEEK 4			
GENTLE COLONY	17	19	0.13 *n.s.*
DEFENSIVE COLONY	16	15	
WEEK 5			
GENTLE COLONY	12	4	1.50 *n.s.*
DEFENSIVE COLONY	4	4	

TABLE V. The number of marked drones drifting away from their home colony are presented with respect to drone genotype and with respect to the defence behaviour of the colony to which they drifted. Contingency table analyses compare the relative numbers of drifting drones of each genotype to each type of colony. Analyses are performed separately for each week as there is non-independence of data between weeks. Degrees of freedom = 1 for all analyses.

The extensive movement of drones between colonies of honeybees has been frequently documented (*e.g.* CURRIE, 1987), though the relationship between drone movement and a colony's traits has rarely been investigated. Drones drifted away from defensive (European) home colonies earlier in their lives than from gentle (European) home colonies. In addition, black drones (carriers of defensive genes) moved away from their home colonies earlier in their lives than yellow drones. But both black and yellow drones preferentially moved into gentle colonies. The data therefore suggest that both colony response (eviction or acceptance of drones) and drone behaviour (drifting or non-drifting) influence the movement of drones, though the precise behavioural mechanisms that are involved are not clear. A more rapid maturation of black drones and of drones in defensive colonies would result in their earlier drifting relative to yellow drones or to drones in gentle colonies.

The number of drones that a colony produces is regulated by a negative feedback mechanism; drones inhibit further drone production (RINDERER *et al.*, 1985 ; FREE, 1987). Hence the differential drifting of drones from defensive colonies to gentle colonies and the differential drifting at a younger age of black drones relative to yellow drones will lead to a suppression of the production of yellow drones (carriers of gentle genes). In a mixed population of gentle and defensive colonies and where there is random mating of queen honeybees, drifting drones will result in the spread of defensive genes. The differential movement of drones from highly defensive AHB colonies to gentle EHB colonies has been documented in Venezuela (RINDERER *et al.*, 1985) and this process of social reproductive parasitism is thought to have contributed to the rapid spread of AHBs in South America. The same effect is seen at Cardiff, though the degree of parasitism of gentle colonies is lower than in South America. It suggests that defence *per se* influences the differential movement of drones.

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