

Dominance orders, worker reproduction, and queen-worker conflict in the slave-making ant *Harpagoxenus sublaevis*

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Received November 20, 1987 / Accepted June 24, 1988

Summary. In a queenright colony of the monogynous slave-making ant *Harpagoxenus sublaevis*, a subset of workers formed a linear dominance order in which dominance was correlated with ovarian development, frequency of trophallaxis, length of time spent in the nest, but not body size. Identical dominance orders occurred in queenless colonies. Experiments in which the top-ranking workers were removed from queenless colonies demonstrated that worker dominance behaviour inhibits egg-laying in subordinates. A similar removal experiment showed queens restrict dominance behaviour and egg-laying in workers, probably pheromonally. Observations of slave raids indicated ovary-developed workers spent significantly less time scouting for slaves, and tended to participate less in slave raids, than workers without ovarian development. These findings suggest that potentially fertile *H. sublaevis* workers aggressively compete for egg-laying rights, consume extra food for egg development, and safeguard their reproductive futures by avoiding risks outside the nest. Hence worker reproduction in this species strongly influences the colony's social structure, nutrient flow, and division of labour, even though all workers in a colony are full sisters. I hypothesize that worker reproduction was formerly even more prevalent in *H. sublaevis*, with workers following the strategy of raising sisters and producing sons predicted by kinship theory. Its continued existence despite queen opposition conceivably results from selection on orphaned workers to reproduce, and the inability of slave-maker workers to raise female-biased broods. The social organization of *H. sublaevis* therefore highlights the importance both of worker reproduction and of the concomitant queen-worker conflict over male parentage in Hymenopteran social evolution.

Introduction

Hamilton (1964) invoked asymmetries in relatedness between close kin caused by haplodiploid sex determination to account for Hymenopteran worker sterility. In social Hymenoptera, full sisters are more closely related to each other (relatedness coefficient, $r=0.75$) than to daughters ($r=0.5$) and less closely related to brothers ($r=0.25$) than to sons ($r=0.5$). Kinship theory therefore predicts that workers under a single, once-mated queen should either (1) reject personal reproduction and rear a female-biased brood of the queen's reproductive daughters and sons, or (2) retain reproductive capability and raise an evenly-balanced brood of the queen's daughters and worker-produced sons. Queens oppose both options, in (1) because queens prefer equal investment in their sexual offspring ($r=0.5$), in (2) because queens favour their own over the less closely related workers' sons ($r=0.25$) (Hamilton 1964, 1972; Trivers and Hare 1976).

Much subsequent work on kinship theory concentrated on the non-reproductive worker option (1), because this part of kinship theory was such an original and plausible solution to the evolutionary puzzle of worker sterility. However, several recent developments suggest the reproductive worker option (2) deserves renewed attention. First, a number of authors have concluded from various models that a worker caste would have evolved more easily if early workers produced males (Aoki and Moody 1981; Iwasa 1981; Bartz 1982; Pamilo 1984). Second, empirical studies reveal worker reproduction is widespread even among advanced social Hymenoptera (Fletcher and Ross 1985; Bourke, in press). Third, evidence exists that queen-worker conflict over male parentage is a ma-

jour feature of Hymenopteran social evolution (West-Eberhard 1981). For all these reasons (see also Bourke, in press), the question of how far Hymenopteran social organization is shaped by selection for worker reproduction is assuming importance in social insect biology (Moritz and Hilleheim 1985; Cole 1986).

In this paper I describe a study of the slave-making ant *Harpagoxenus sublaevis* concerning the influence of worker reproduction on worker behaviour, queen regulation of worker fertility (queen control), and temporal division of labour. *Harpagoxenus* species are obligate slave-making social parasites of *Leptothorax* ants (Buschinger et al. 1980; Buschinger 1981). Queens invade *Leptothorax* nests, then produce workers which raid neighbouring *Leptothorax* colonies for brood. The *Leptothorax* workers emerging from captured brood (slaves) perform all nursing and foraging for the slave-makers. The biology of the European *H. sublaevis* has been extensively studied by A. Buschinger et al. (e.g. Buschinger 1966a, 1966b, 1968; Winter 1979). Each colony contains at most one maternal slave-maker queen (monogyny), who is singly-mated (Buschinger and Alloway 1979; Bourke et al. 1988). Workers possess ovaries but no spermatheca, and hence can produce males parthenogenetically: dissections reveal egg-laying workers occur in both queenright and queenless (orphaned) colonies (Buschinger and Winter 1978; Bourke et al. 1988). To increase their inclusive fitness, *H. sublaevis* workers can therefore either bear sons, or indirectly help rear kin by raiding for slaves. Since fertile workers should refrain from raids because this hazardous activity jeopardizes their personal fitness, one means of examining the reproductive tactics of slave-maker workers is to investigate their behaviour on slave raids.

I report the discovery that in both queenright and queenless colonies potentially fertile *H. sublaevis* workers form competitive, linear dominance orders. Similar orders have previously been found in only two other ant species (*Leptothorax allardycei*, Cole 1981, 1986; *H. americanus*, Franks and Scovell 1983). I test the hypothesis that worker dominance behaviour inhibits rival reproductive activity, and that *H. sublaevis* queens oppose worker reproduction. Franks and Scovell (1983) found high ranking *H. americanus* workers never scouted in search of slave colonies to raid. I test the hypothesis that potentially fertile *H. sublaevis* workers participate reluctantly in both scouting and slave-raiding. A companion paper (Bourke et al. 1988) deals with genetic and demographic issues associated with worker reproduction in

H. sublaevis, including the level of worker male production, frequency of fertile workers, intra-colony relatedness, colony productivity, and sex investment ratio.

Methods

Field collections

In June–July 1983–1985 collections of slave-maker colonies were made from coastal pinewoods between Bröms and Kristianopel, Blekinge, S.E. Sweden, and from an inland site at Önnarp, near Röke, Skåne, S. Sweden. In both localities colonies of *H. sublaevis* and its *Leptothorax* slave species occur in dead twigs on the ground. Single *H. sublaevis* colonies occupy single twigs (monodomy). Whole colonies were therefore collected by fragmenting twigs and aspirating the ants and brood. Six colonies were selected from the field collections for experimental study (Table 1). Colonies 1–2 and 4–6 came from the Bröms-Kristianopel population, and colony 3 from the one at Önnarp.

Culture methods

Colonies were maintained in laboratory incubators adjusted to simulate annual climatic cycles (Buschinger 1973, 1974, personal communication), and studied in their first or second artificial summer after collection. Each colony was housed in a nest made of two 5 × 7.5 cm plain glass slides separated by a cardboard wall. Each nest rested in a clear plastic box containing a drinking water supply (water tube stoppered with damp cotton wool), a humidifier (gauze covered water-tray), artificial ant diet (Bhatkar and Whitcomb 1970), and (except in colony 1) fresh insect food (*Drosophila* larvae).

Observation methods and conditions

All slave-makers were individually marked, in colony 1 with coloured paints, in colonies 2–6 with 0.65 × 0.85 mm paper letters glued to the thorax. Paper letters lasted longer than paint marks and individually characterized ants on black and white video recordings.

Colonies 1–4 were each observed over 3–5 weeks for a total of nearly 140 h in a series of separate (approximately daily), standardized one hour observation bouts. In each bout I

Table 1. Adult composition of 6 experimental *H. sublaevis* colonies

Colony no.	Number of individuals			
	<i>H. sublaevis</i> maternal (mated, egg-laying) queen	<i>H. sublaevis</i> workers	<i>H. sublaevis</i> non-laying queens	<i>L. acervorum</i> slaves
1	1	14	0	85
2	0	6	0	39
3	0	7	4	19
4	1	14	4	37
5	1	13	6	50
6	1	22	0	34

watched an entire colony through a binocular microscope. Each colony was illuminated by a cold light source and maintained at 25–27° C by a heated stage, except colony 1 (unheated, average temperature 21.3° C). All observations took place with colonies in their artificial summer phase to coincide with egg-laying, except the first 15 h observation of colony 1, which took place during artificial springtime. All observations were made in daytime, and at least 30 mins acclimatization was allowed between transferring the colony to the microscope stage and starting observations.

Definitions of behaviours

In each observation bout in colonies 1–4 I recorded every occurrence of dominance, aversion and trophallaxis involving slave-makers. Dominance took two forms. In the severe form a slave-maker bit and gripped another's appendage (e.g. leg, antenna) for a few seconds to several minutes. In the milder form a slave-maker rapidly approached and either antennated or temporarily climbed on top of another. Attacking ants frequently flexed their gasters towards those under attack as if to sting them, although they never actually protruded their stings. Ants under attack never defended themselves but typically withdrew their antennae and remained still. Attacks ended with the release of the attacked by the attacking ant.

Aversion (or avoidance: Franks and Scovell 1983) occurred when a slave-maker recoiled violently from another (higher ranking) slave-maker following antennal contact. Trophallaxis (solicitation of liquid food) from slaves or larvae is probably the sole means by which *Harpagoxenus* workers obtain nourishment, since they rarely if ever forage for food (Stuart and Alloway 1985).

I also recorded the amount of time each slave-maker spent outside the nest in the nest box arena, and in colonies 1 (every 15 min) and 4 (every 5 min) the identity of the slave-maker nearest the egg-pile.

Video recording

To observe egg-laying, activity in colony 4 was video recorded for a total 229.8 h in 13 separate sessions between observation bouts. The colony was filmed with a Panasonic TV camera mounted on a Zeiss binocular microscope, and recordings made with a Panasonic Time Lapse Video Recorder. Temperature and photoperiod matched those in incubators in the artificial summer phase, except that at night temperature was uncontrolled (room temperature was ca. 18° C) and the colony was illuminated by an infra-red source, to permit night filming (infra-red light is invisible to ants).

Experimental treatments

Colony 1 (queenright) was observed for 30 h over 3 weeks to determine basic social structure.

Colonies 2 and 3 (both queenless) were each observed for 36 h over 5 weeks to determine social structure in orphaned colonies, and to study effects of removing the top-ranking worker. I therefore observed each colony for 12 h with the top-ranking worker present, 12 h with the worker removed, and 12 h with the worker returned (control). During their isolation period, each top-ranking worker was kept with two slaves, food and water. Daily fluctuations in egg number inside both colonies 2 and 3 were recorded throughout the experiments to infer the identity of layers.

Colony 4 (queenright) was used to test for queen inhibition of worker fertility (queen control) in an experiment with the

same design as the worker removal experiments (12 h observation when queen present, 12 h when removed, 12 h when returned). Isolation conditions for the queen when removed were the same as for the top-ranking workers in colonies 2 and 3. In addition, before the first observations of colony 4 the queen was isolated for 5 days in a dish containing the dye Fat Red, to stain her eggs (by ingestion). All other colony 4 slave-makers were simultaneously isolated in dye-less dishes to minimize effects of the queen's absence prior to the experiment. After observation bout 24, egg-laying in colony 4 was also video recorded (details above).

Colonies 5 and 6 (both queenright) were used to investigate individual differences between slave-makers in scouting (searching for slaves) and slave-raiding. Slave raids were induced following Winter's (1979) split arena technique. During their artificial summer each colony was placed for 6–7 days in a large (48 × 48 × 8 cm high) sand-filled arena separated by a removable barrier from a similar arena containing a colony of *Lep-tothorax acervorum* (slave species). Daytime temperatures were 22–28° C. Over 6–7 days before raiding, scouting by slave-makers was recorded in 6 daily 2 h bouts. A slave-maker was considered to be scouting on leaving a 10 × 10 cm area around the slave-maker nest. At ca. 1400 h on the 6th or 7th day in the arena, a slave raid was induced by removing the barrier separating the slave-maker from the *L. acervorum* colony. The behaviour of individual slave-makers was continuously monitored for the duration of each raid.

Brood removal and colony size manipulation

In all colonies except 1 and 2 slave-maker brood was removed before (or shortly after) the first observations and replaced with equivalent amounts of *L. acervorum* brood, to prevent new *H. sublaevis* females reaching adulthood during the study period. In some ants, the presence of conspecific brood inhibits worker fertility (Dartigues and Passera 1979; Smeeton 1982). However, brood removal did not account for the results of this study since workers in colony 1 exhibited ovary development even though the brood was not replaced in this colony, and conversely worker egg-laying activity only appeared in colony 4 after the queen's removal, and then ceased when she was returned, although the brood was replaced before the start of observations.

In colonies 5 and 6 alone the numbers of adult slave-maker females were artificially reduced ca. 4 weeks before scouting recordings began, because previous numbers were too high to allow simultaneous observation of all ants, and suitable queenright colonies were otherwise unobtainable. In colony 5 the slave-maker population was reduced from 33 females to 20, in colony 6 from 41 to 23 (Table 1). Excluded females were arbitrarily chosen, except I ensured the colony queen remained in each colony.

Ovarian dissections and size measurements

At the end of each experiment slave-makers from all colonies were dissected to determine their caste and reproductive status. The ovaries were removed in Ringer's solution with fine forceps, and the numbers of active ovarioles, oocytes, and corpora lutea (ovariolar structures indicating egg-laying activity) were counted under a compound microscope (dissection method after Buschinger and Alloway 1978). In Swedish *H. sublaevis* populations queens are exclusively wingless and morphologically externally indistinguishable from workers (ergatoidy: Buschinger and Winter 1975). Such queens could be identified by the spermatheca, visibly full of sperm if queens were mated.

The maximum pronotal width of each slave-maker was also measured as an index of body size.

The ovarian dissections revealed that colonies 3-5 contained unmated, non-laying queens (Table 1) despite having been given the opportunity to release sexuals following capture. In nature almost all young *H. sublaevis* queens leave the maternal nest in the year of production (Buschinger and Winter 1978). The lingering presence of young queens in colonies 3-5 at relatively high frequencies was therefore unnatural. However, these queens did not appear to perturb colony organization, since they never exhibited dominance behaviour and high ranking workers treated (and dominated) them apparently like passive workers.

Results

Worker dominance order in a queenright colony

In colony 1 a subset of 3 slave-maker workers behaved aggressively towards the remaining 11, non-aggressive slave-maker workers (Table 2). The queen and the 3 aggressive workers formed a sta-

ble, linear dominance order headed by the queen (0.9% of aggressive interactions involved rank reversals). Dominance behaviour was correlated with the following: 1) Ovarian development. All 3 aggressive workers had ovarian development, compared to only 1 of the 5 passive workers dissected (One-tailed Fisher's exact test, $P=0.07$). 2) Frequency of trophallaxis. The aggressive workers solicited trophallaxis from slaves or larvae at a mean rate of 0.89 times/h, compared to 0.51 times/h in passive workers (One-tailed Mann-Whitney U-test, $U_{11,3}=28, P=0.05$). The queen had the greatest rate of trophallaxis (2.27 times/h) and fed disproportionately more often from larvae than workers (0.24 of the queen's trophallaxis involved larvae, compared to 0.15 of the workers'). Trophallaxis between slave-makers was extremely rare (0.7% of all their trophallaxis), as was interference by slave-makers with other slave-makers' trophallaxis

Table 2. Worker dominance order and correlates of dominance in the queenright colony 1 (30 h observation). The upper part of the table shows the total number of dominance interactions between given pairs of ants (both forms of dominance pooled), followed in brackets by the total number of aversions. Q was the colony queen, and ants RY to R were all workers. In the lower part of the table (correlates of dominance) blanks (-) indicate information lacking due to loss of paint marks

	Subordinate (Averting ant)															Total times dominating	Total times averted
	Q	RY	OP	OR	O	P	GR	PY	GP	GY	GO	PR	Y	G	R		
Q	-	0 (7)	1 (5)	0 (2)		1 (4)				0 (2)					1	3	(20)
RY		-	10 (27)	0 (3)		2	1	1		3	0 (1)	2	2	1	2 (1)	24	(32)
OP		1	-	7 (6)	3	2	5 (1)	6		7	5	8 (1)	7	11	11	80	(8)
OR				-	1					1		1	2	1	4	10	(0)
Dominant (Ant averted)																	
O					-											0	(0)
P			0 (1)			-										0	(1)
GR							-									0	(0)
PY								-								0	(0)
GP									-							0	(0)
GY										-						0	(0)
GO						0 (1)					-					0	(1)
PR												-				0	(0)
Y													-			0	(0)
G														-		0	(0)
R															-	0	(0)
Total times dominated	0	1	11	7	4	5	6	7	8	8	9	9	11	13	18	117	
Total times averting	(0)	(7)	(33)	(11)	(0)	(5)	(1)	(0)	(0)	(2)	(2)	(0)	(0)	(0)	(1)		(62)
Trophallaxis rate/h	2.27	1.03	0.85	0.79	0.39	1.00	0.14	0.45	0.66	0.37	0.31	0.12	0.44	1.16	0.55		
Mean time (min) outside nest/h	0	0	3.5	12.0	14.0	2.2	16.2	18.6	14.7	6.0	15.3	10.0	14.8	10.2	2.0		
Pronotal width (mm)	0.65	0.60	0.60	0.62	0.57	-	0.62	-	0.57	0.60	0.62	0.59	-	0.60	-		
No. active ovarioles	6	5	6	1	-	-	-	-	0	0	0	0	-	1	-		
No. oocytes	17	10	12	1	-	-	-	-	0	0	0	0	-	1	-		
No. corpora lutea	5	2	1	1	-	-	-	-	0	0	0	0	-	3	-		

Table 3. Worker dominance order and correlates of dominance in the queenless colony 2 (initial 12 h observation). All ants were workers. Ovarian dissections were performed after removal then replacement of the top-ranking worker (R), except in the case of D, who died from unknown causes after observation bout 14

		Subordinate (Averting ant)						Total times dominating	Total times averted
		R	A	B	D	E	F		
Dominant (Ant averted)	R	–	53 (18)	17	21 (1)	24 (4)	25 (1)	140	(24)
	A		–	17 (1)	29	26	40 (1)	112	(2)
	B to F			–				0	(0)
Total times dominated		0	53	34	50	50	65	252	
Total times averting		(0)	(18)	(1)	(1)	(4)	(2)		(26)
Trophallaxis rate/h		2.92	2.70	1.20	0.52	1.76	0.25		
Mean time (min) outside nest/h		0	0.7	18.3	11.5	0.2	0.8		
Pronotal width (mm)		0.57	0.52	0.62	0.56	0.61	0.47		
No. active ovarioles		6	4	0	0	0	4		
No. oocytes		14	6	0	0	0	5		
No. corpora lutea		3	4	0	0	0	0		

(0.9% of slave-maker/slave trophallaxis resulted from one slave-maker interrupting another). 3) Time outside the nest. Only the queen and the top-ranking aggressive worker never left the nest. Aggressive workers on average left the nest for 5.2 min/h, whereas passive workers were outside 11.3 min/h, although this difference was not significant (One-tailed Mann-Whitney U-test, $U_{11,3} = 26$, $P = 0.1$).

The queen was the slave-maker nearest the eggs for 85% of all records (n records = 96), far greater than the expectation based solely on the amount of time the queen spent in the nest relative to the other slave-makers ($\chi^2_1 = 746.7$, $P < 0.001$).

Size did not appear to be a correlate of dominance. The mean pronotal widths of aggressive and passive workers were 0.61 and 0.60 mm respectively ($t_8 = 0.840$, $P > 0.1$).

These results suggested that in a queenright *H. sublaevis* colony potentially fertile *H. sublaevis* workers (1) inhibit their prospective rivals' ovarian development with aggressive dominance behaviour, (2) consume extra food for egg development, and possibly (3) protect their reproductive futures by avoiding risks outside the nest.

Worker dominance orders in queenless colonies, and effects of removing top-ranking workers

In both queenless colonies (nos. 2 and 3) worker dominance orders existed as in the queenright colony 1 (Tables 3 and 4). The numbers of aggressive workers in the two colonies were 2 and 3 respectively. Correlates of dominance (ovarian development, frequency of trophallaxis, time outside the

nest) were the same as in colony 1, except that in colony 3 for unknown reasons the passive workers had a higher rate of trophallaxis. Thus, in colony 2, the mean trophallaxis rate of aggressive workers was 2.81 times/h, compared to 0.93 times/h in passive workers. The mean time outside the nest in aggressive workers was 0.35 min/h, and in passive ones 7.7 min/h (Table 3). In colony 3 the mean trophallaxis rates of aggressive and passive workers were 1.29 and 1.69 times/h respectively, and the mean times outside the nest 2.5 and 27.0 min/h respectively (Table 4). As in colony 1, all aggressive workers in colonies 2 and 3 were ovary-developed, and there was only one passive, ovary-developed worker per colony (Tables 3 and 4). Therefore, considering colonies 2 and 3 together, all 5 aggressive workers had ovarian development, compared to 2 out of 8 passive workers, indicating a significant association between worker dominance behaviour and ovarian development (One-tailed Fisher's exact test, $P = 0.02$). Finally, for unknown reasons, the level of aggression varied greatly in colonies 1–3 (the numbers of dominance acts per aggressive ant per hour were 0.47 in colony 1, 5.28 in colony 2, 0.36 in colony 3).

In both colonies 2 and 3 immediately after the top-ranking (alpha) worker was removed, the egg count stopped rising. Both alpha workers laid eggs in isolation, suggesting they were initially the sole layers in their respective colonies. Within 5 (colony 2) and 6 (colony 3) days of the alpha worker's removal, the egg count in both colonies started rising again. Since in both colonies later dissection revealed the only other slave-maker apart from the alpha to possess corpora lutea was the second-

Table 4. Worker dominance order and correlates of dominance in the queenless colony 3 (initial 12 h observation). All ants were workers except V, H, S, and K, which were non-laying queens (see Methods). Ovarian dissections were performed after removal then replacement of the top-ranking worker (L), except in the case of I, who like a single worker in colony 2 (Table 3) died during the experiment from unknown causes (after observation bout 4)

		Subordinate (Averting ant)										Total times dominating	Total times averted	
		L	J	T	I	B	V	H	S	N	K			Z
Dominant (Ant averted)	L	–	3 (14)	1 (3)		1	1 (1)	2	1	6 (1)	9 (3)	8	32	(22)
	J		–						1	2	1	4	(0)	
	T			–							1	1	(0)	
	I to Z				–							0	(0)	
Total times dominated		0	3	1	0	1	1	2	2	8	9	10	37	
Total times averting		(0)	(14)	(3)	(0)	(0)	(1)	(0)	(0)	(1)	(3)	(0)		(22)
Trophallaxis rate/h		1.0	1.27	1.59	1.71	1.29	0.34	0.72	1.28	1.65	0.85	2.11		
Mean time (min) outside nest/h		0	0.9	6.6	33.8	52.3	15.3	53.1	13.1	2.3	6.8	19.7		
Pronotal width (mm)		0.47	0.53	0.52	0.52	0.59	0.57	0.57	0.57	0.59	0.57	0.60		
No. active ovarioles		6	6	7	0	0	0	0	1	4	0	0		
No. oocytes		7	22	12	0	0	0	0	1	4	0	0		
No. corpora lutea		4	2	0	0	0	0	0	0	0	0	0		

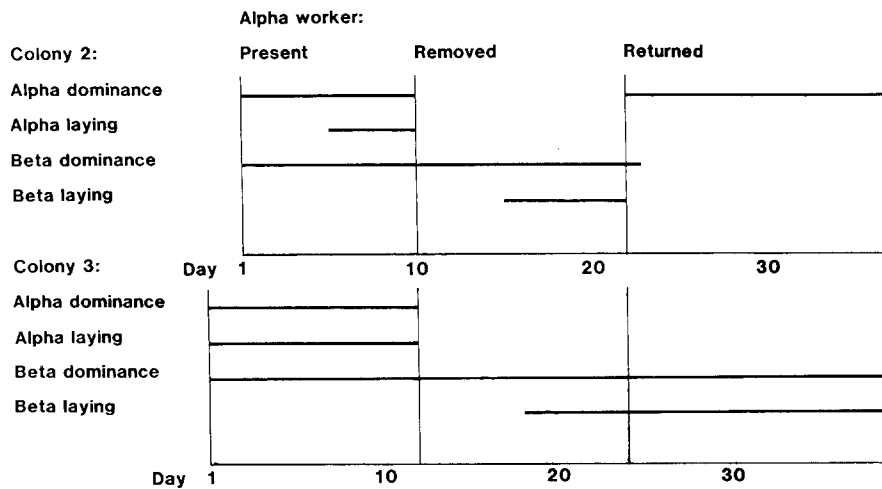


Fig. 1. Effects of removing then replacing the top-ranking worker in queenless colonies (colonies 2 and 3: 36 h observation each)

ranking (beta) worker (Tables 3 and 4), the new egg-layer following alpha's removal must have been beta in both cases. In colony 3 beta was in fact seen laying an egg 6 days after alpha's removal.

The results of returning the alpha worker differed in the two colonies (Fig. 1). In colony 2 the newly-returned alpha attacked the former beta, and thereby resumed its top-ranking position. Beta ceased both dominance behaviour and egg-laying within a day of alpha's return. In colony 3 the newly-returned alpha was itself attacked by the former third-ranking (gamma) worker (risen to the beta position in alpha's absence). Over the following days alpha, like a passive ant, exhibited neither

dominance behaviour nor laying (as confirmed by the absence of ripe eggs in its ovaries in the final dissections). Beta, by contrast, continued to show dominance behaviour and was also observed egg-laying.

The fact that in both colonies the beta worker started laying eggs following alpha's removal, and that on alpha's return each beta ceased or continued laying according to whether alpha assumed a higher or lower rank, confirmed that in *H. sublaevis* workers dominance behaviour inhibits egg-laying in subordinates. The reason for the (instructive) failure of the alpha worker to regain its top-ranking position in colony 3 was unknown.

As in colony 1, large size was not a correlate

of dominance in colonies 2 and 3. The mean pronotal widths of the aggressive and passive ants were respectively 0.55 mm ($n=2$) and 0.57 mm ($n=4$) in colony 2, and 0.51 mm ($n=3$) and 0.58 mm ($n=4$) in colony 3 (Tables 3 and 4).

Effects of queen removal

In the first 12 h observation of colony 4 worker (and queen) dominance behaviour was totally absent. All eggs laid were dyed, indicating the queen was sole layer at this time. When the queen was removed one worker (J) began to show dominance behaviour within 24 h. A second aggressive worker arose 12 days after the queen's removal. Within 8 days of the queen's removal J began egg-laying. Video recordings and later dissection showed J to be sole layer in the queen's absence. When the queen was returned, J ceased laying in about 2 days, but continued with dominance behaviour. The queen therefore again became the colony's only egg-layer.

Initially the queen in colony 4 occupied the position nearest the egg-pile for 82% of records (n records = 134). This figure was very close to that recorded for the queen of colony 1 (see above), and again far greater than expected just on the basis of the relative amount of time the queen was in the nest ($\chi^2_1 = 1383.1$, $P < 0.001$). As in colony 1, the queen had the greatest frequency of trophallaxis (1.67 times/h compared to the average worker trophallaxis frequency of 0.48 times/h), and fed more often from larvae (0.65 of the queen's trophallaxis was from larvae, compared to 0.03 of the workers'). In the queen's absence the laying worker J adopted the position nearest the eggs for 55% of records, a figure far higher than the corresponding figures for the previous and following periods when the queen was present (J nearest eggs for 2% and 5% of records respectively) (Total n records = 403, $\chi^2_2 = 146.1$, $P < 0.001$). J's rate of trophallaxis increased from 0.26 times/h to 1.75 times/h on the queen's removal, and fell to 0.85 times/h on the queen's return. J only conducted trophallaxis with larvae (0.21 of J's trophallaxis) during the queen's absence.

These results suggested that the *H. sublaevis* queen inhibits worker dominance behaviour and egg-laying. Queen control is presumably mediated pheromonally, since in colony 4 the queen was never aggressive, and in colony 1 she was only slightly aggressive (Table 2). The results also showed that laying workers, like queens, characteristically remain close to the egg-pile, and furthermore confirmed that laying workers display an in-

creased rate of trophallaxis. Trophallaxis with larvae appeared to be associated with egg-laying.

Worker fertility and scouting and raiding behaviour

In both colonies 5 and 6 the overall level of worker ovarian development was low, the mean worker oocyte number being 4.7 in colony 5 and 2.9 in colony 6 (Table 5), compared to 6.0, 8.3 and 9.2 in colonies 1, 2 and 3 respectively (Tables 2, 3, 4). Despite this, in each colony ovary-developed workers spent significantly less time scouting than workers without ovarian development (Table 5). This difference was greater in colony 5 than in colony 6, matching the greater ovarian development of colony 5 workers relative to colony 6 workers. Therefore, the prediction ovary-developed workers should avoid risks, possibly great in scouting since scouting is a solitary activity (Buschinger et al. 1980; Franks and Scovell 1983), was fulfilled.

The two slave raids followed the sequence – discovery of *Leptothorax* nest, recruitment, fighting, brood transport – typical for *H. sublaevis* (Winter 1979; Buschinger et al. 1980). In both colonies every slave-maker except the two colony queens, who remained in their nests throughout, took part in the raids. However, the average degree of participation by the ovary-developed workers was less than that of non-ovary-developed workers, in the following ways. 1) Response time to recruitment. In *H. sublaevis*, successful scouts return to their home nest and recruit nestmates with an intranidal excitation display followed by a pheromonal invitation to a partner to follow the scout in tandem to the raid target nest (Buschinger and Winter 1977; Buschinger et al. 1980). In both colonies 5 and 6, ovary-developed workers present in the slave-maker nest when the first recruitment occurred, on average entered the *Leptothorax* arena later than the non-ovary-developed workers also present in the nest at that time (Table 5). Though this difference was small in colony 6 and not significant in either colony, in colony 5 chiefly because of small worker numbers (One-tailed Mann-Whitney U-tests, both $P > 0.1$), this finding implied a reluctance to respond to recruitment to the raid target on the part of the ovary-developed workers.

Ovary-developed workers also differed in (2) Adoption of specialist roles. Considering both colonies together, 12 of the combined population of 35 slave-maker workers (comprising 19 non-ovary-developed and 16 ovary-developed workers: Table 5), acted as tandem leaders or brood trans-

Table 5. Differences between slave-maker workers with and without ovarian development (OD, i.e. oocytes present) in scouting and raiding behaviour (colonies 5 and 6). Two colony 5 workers without OD possessed corpora lutea. Raid phases were defined as follows: (1) Time from barrier removal to first tandem recruitment; (2) First to last tandem recruitment; (3) First slave-maker entry into *Leptothorax* nest to expulsion of all occupants; (4) First to last brood transport

No. workers		Pronotal width (mm) (SD)		Souting results Mean time (min)/h scouting (SD)		Raids results					
Without OD	With OD (Mean no. oocytes) (range)	Workers without OD	Workers with OD	Workers without OD	Workers with OD	Duration (min) from start of raid of:		Mean time (min) from first recruitment to entry to target arena in workers present at first recruitment			
						(1) Time to first recruitment	(2) Tandem recruitment	(3) Fighting in target nest	(4) Brood transport	Workers without OD (n)	Workers with OD (n)
Colony 5											
7	6 (4.7) (1–9)	0.55 ^a (0.03)	0.55 ^a (0.01)	26.7 ^c (9.9)	5.6 ^c (4.8)	(1) 0–13	(2) 13–233	(3) 41–102	(4) 122–233	20 (2)	94 (5)
Colony 6											
12	10 (2.9) (1–9)	0.55 ^b (0.02)	0.56 ^b (0.02)	14.2 ^d (11.8)	9.5 ^d (13.7)	(1) 0–14	(2) 14–414	(3) 43–59	(4) 147–428	46 (7) ^e	53 (5)

^a NS ($t_{11}=0.138$, $P>0.1$); ^b NS ($t_{20}=0.055$, $P>0.1$); ^c $P<0.001$ (t -test with bout data pooled to homogenize variances, $t_{11}=4.737$); ^d $P<0.05$ (d -test with unpooled bout data, $d=2.102$); ^e Figure excludes a single non-ovary-developed worker not entering *Leptothorax* arena

porters. Eight were non-ovary-developed and 4 had ovarian development. Therefore, fewer ovary-developed workers adopted these specialist roles than expected on the basis of their relative abundance, though this difference was not significant ($\chi^2_1=0.76$, $P>0.1$). 3) Involvement in fights. Of 56 separate fights between slave-makers and hostile *Leptothorax* recorded in the two raids, 34 involved non-ovary-developed workers and 22 workers with developed ovaries. Therefore ovary-developed workers took part in fewer fights than their relative abundance suggested, though again the difference was not significant ($\chi^2_1=0.93$, $P>0.1$). A single slave-maker was killed by hostile *Leptothorax* in the raids, a non-ovary-developed worker from colony 6.

These findings, although not individually conclusive, were all as predicted by the hypothesis that prospectively reproductive *H. sublaevis* workers should avoid the risks associated with slave-raiding. To this extent, the hypothesis was confirmed. A possible reason for the fact no ovary-developed worker avoided raiding totally was the low average level of ovarian development among such workers in both colonies (see above and Table 5). The reduction of slave-maker number prior to observations in both colonies (see Methods) could also have had a greater disruptive effect on colony organization than anticipated.

In agreement with previous results, size was not a correlate of ovarian development or scouting and raiding behaviour in colonies 5 and 6 (Table 5).

Discussion

This study shows *H. sublaevis* workers form competitive dominance orders in which high-ranking, ovary-developed individuals inhibit egg-laying in subordinates by means of physical aggression. *H. sublaevis* is only the third ant species in which such orders have been found (see Introduction), though similar dominance systems are relatively common among social wasps and bees (Wilson 1971; Fletcher and Ross 1985; Bourke, in press). This study further demonstrates that *H. sublaevis* queens inhibit worker reproductive activity, almost certainly pheromonally. Such an ability is also matched by queens of other social insects (Wilson 1971; Brian 1980). Finally, the results confirm that worker reproduction constrains the temporal division of labour (Wilson 1985).

Wilson (1971: 334) suggested that in some wasp species dominance systems do not reflect intra-colony reproductive competition but instead serve to promote a more – not less – efficient division of labour at colony level. Similarly, in *H. sublaevis*, the system conceivably acts as a colony-level mechanism for determining which slave-makers should

raid, since it is probably disadvantageous from the colony's viewpoint for all slave-makers to raid (and risk death) simultaneously. However, several lines of evidence contradict this hypothesis. First, the hypothesis does not explain the lack of dominance activity in some queenright colonies (e.g. colony 4). Second, the hypothesis arguably predicts that worker size should be correlated with division of labour, since larger workers presumably make better raiders. But no such correlation exists (Table 5). Third, dominance behaviour is costly to the colony because of the increased trophallaxis rate of the aggressive slave-makers. Although there is no clear evidence that worker dominance activity reduces colony productivity (see Bourke et al., in press), these facts make it unlikely that the dominance system enhances efficiency.

Commonly, reproductive worker social insects lay eggs when young and switch to risky colony-beneficial tasks when old. In this way they change their reproductive tactics according to their diminishing chances of future survival as senescence approaches (Wilson 1985). Though they were not aged in this study, reproductive *H. sublaevis* workers may undergo this change, since Buschinger et al. (1980) found *H. sublaevis* scouts were at least one year old. However, it seems unlikely all *H. sublaevis* workers are reproductive when young, because if they were a greater proportion of workers with corpora lutea but without ovarian development would occur than was found (colonies 1–6 contained only two such workers, from colony 5: Table 5).

The social structure of *H. sublaevis* colonies closely resembles the competitive dominance system in *H. americanus* discovered by Franks and Scovell (1983). One difference is that in *H. americanus* all individuals are aggressive, including the queen. Pheromonal rather than behavioural queen control in *H. sublaevis*, and the existence of a passive subset of workers, could stem from the larger colony size of this species. Another difference is that in *H. americanus* dominance interactions frequently involved disputes over trophallaxis, whereas in *H. sublaevis* dominance arose seemingly spontaneously. Most intriguingly, both dominance orders and slave-making almost certainly evolved independently and convergently in *H. americanus* and *H. sublaevis*. *H. americanus* is no longer considered a true congener of the other *Harpagoxenus* species, but most probably arose from a separate, non-parasitic leptothoracine stock (Buschinger 1981).

Electrophoretic allozyme analysis of *H. sublaevis* from the Bröms-Kristianopel population indi-

cates that in each colony the queen is singly-mated (Bourke et al. 1988). Hence all slave-maker workers in a colony are full sisters. Confirming this, the estimated regression coefficient of relatedness (\pm SE) between female colony members was 0.735 ± 0.044 , which was not significantly different from the maximal 0.75 value attainable in outbred social Hymenoptera (Bourke et al., in press). With this (for ants) unusual background of a combined knowledge of social and genetic colony structure, I now discuss the significance of *H. sublaevis* worker reproduction for kinship theory.

Clearly, worker reproduction in *H. sublaevis* strongly influences individual behaviour, colony nutrient flow, and division of labour. But given maximal intra-colony relatedness in *H. sublaevis*, the high observed degree of worker "selfishness" is unexpected, assuming *H. sublaevis* workers are striving to follow kinship theory's sterile worker option (see Introduction). The question therefore arises as to why *H. sublaevis* workers are so "selfish". In answer I propose, first, that worker reproduction was formerly even more prevalent in *H. sublaevis* because workers, instead of adopting sterility, followed the alternative, reproductive worker option predicted by kinship theory of raising sisters and producing sons (see Introduction). Second, I suggest that in response queens developed increasingly effective power to inhibit worker laying, to the point worker reproduction is now absent in some queenright colonies. Hence the social structure of *H. sublaevis* represents the current state of a kin-selected queen-worker conflict over male parentage.

Since it would be advantageous to every *H. sublaevis* queen to inhibit queenright reproductive activity in her workers, reasons must exist for why this has so far not occurred. I suggest two such reasons (not mutually exclusive), the first of which involves monogyny and colony orphanage. In monogynous species colony orphanage through natural queen mortality is a likely event (30% of *H. sublaevis* colonies in the Bröms-Kristianopel population were queenless: Bourke et al. 1988). Orphanage evidently frees workers with reproductive capability from queen inhibition. In this study the highest level of worker dominance activity occurred in the queenless colony 2. Further, in the field queenless *H. sublaevis* workers are the most commonly fertile, and produce most worker-derived males (Bourke et al. 1988). Therefore, in monogynous species, workers may have been selected to retain reproductive capability because of the high probability, when orphaned, of being able to produce male eggs without queen interference

(see also Bourke, in press). Some of the behaviour of ovary-developed workers in queenright *H. sublaevis* colonies could consequently be for maintaining dominance rank in anticipation of the queen's death, i.e. for future rather than present reproductive gain, thereby partially explaining inferred low worker male production levels in queenright colonies (Bourke et al. 1988). However, the main point here is that workers selected to be poised for reproduction when the queen dies, may also be harder for queens to inhibit in queenright conditions.

A second reason for the high level of *H. sublaevis* worker reproduction concerns the species' parasitic habits and sex investment ratio. The sterile worker option in kinship theory involves workers raising a female-biased brood of the queen's sexual offspring. As corroboration of the theory, in many non-parasitic ant species, sterile workers raise such broods (Trivers and Hare 1976; Nonacs 1986). But in slave-makers, workers – since they are not involved in brood care – almost certainly lack the practical power of non-parasitic workers to manipulate brood composition towards the female-biased sterile worker optimum in the face of opposition from queens, who favour equal investment in their progeny (Trivers and Hare 1976). This presumed lack of slave-maker worker control over sex ratio appears genuine, since approximately 1:1 investment has been found in slave-makers (Trivers and Hare 1976; Nonacs 1986; Bourke et al. 1988).

This situation may promote worker reproduction in slave-makers, because rather than follow the (for them) suboptimal course of helping raise an evenly-balanced queen-produced brood, slave-maker workers could instead pursue the alternative of individual male production. In other words, their inability to raise a female-biased queen-produced brood could explain apparently strong selection on *H. sublaevis* workers to retain their reproductive option.

H. sublaevis is one of numerous social Hymenopteran species with reproductive workers. It seems likely many features of Hymenopteran sociality are best explained by supposing, as in *H. sublaevis*, that within each species worker reproduction was formerly even commoner and that queen-worker conflict over worker reproduction has been a major theme of each species' subsequent social development (West-Eberhard 1981; Bourke, in press). Hence the social structure of *H. sublaevis* confirms that renewed attention should be paid to kinship theory's reproductive worker alternative in future studies of Hymenopteran social evolution.

Acknowledgements. I thank Nigel Franks for help with every aspect of this work. I also thank Per and Steffi Douwes for help and hospitality during field trips to Sweden. Tom van der Have and Alfred Buschinger commented on earlier versions of the manuscript, and Alfred Buschinger also generously made available his leptothoracine culture techniques. For use of its facilities, I acknowledge the Ecological Station of Uppsala University, Öland. This work was supported by a University of Bath Research Studentship. Additional funding came from the Balfour-Browne Fund of the University of Cambridge, and from grants to Nigel Franks awarded by the Royal Society and the Nuffield Foundation.

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