

The length of time that the concentrations of dissolved ions have been elevated is consistent with permeability enhancement as a mechanism to explain the hydrological response to the earthquake. Shales in the region have probably undergone long-term permeability changes. The increase in ionic concentration may be attributed to shale units transporting a significantly greater fraction of ground water to the streams. The data are also consistent with the relatively short-lived (6-month) nature of the stream flow increases associated with the earthquake²; because residence times of ground water in the basin are short, any stream flow augmentation associated with a lowering of the water table can also be expected to be short-lived. Stream chemistry may be dominated by changes in groundwater flow paths, but stream flow is generally governed by the magnitude of recent precipitation.

Hydrological changes associated with other major earthquakes are also compatible with the mechanism of permeability enhancement, but the data sets associated with such earthquakes focus almost entirely on the magnitude of stream flow. Recent work examining stream-flow response to major earthquakes in North America has shown that such processes

as dilatant crack closure⁹ and expulsion of pressurized middle-crustal fluids¹⁰ are not easily reconciled with observations¹. The Loma Prieta data also indicate that such mechanisms are unlikely to produce the observed hydrological changes. Finally, the stream chemistry and groundwater data allow both the mechanisms of seismically induced compression and permeability enhancement to be tested. The mechanism of permeability enhancement provides a more complete explanation of the observations. □

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Unexpected patterns of parentage and relatedness in a primitively eusocial bee

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In species with haplodiploid genetic systems, full sisters are more closely related to each other ($r = \frac{3}{4}$), and less closely related to their brothers ($r = \frac{1}{2}$), than to their daughters and sons ($r = \frac{1}{2}$). The classical theory for the origin of hymenopteran eusociality predicts that in many primitively or facultatively eusocial species, workers should exploit this relatedness asymmetry by laying male-destined eggs while allowing the queen to lay gyne-destined (reproductive female) eggs^{1–3}. This prediction is satisfied in many species where colonies are founded by solitary gynes^{4–8}. Here we describe a surprising reversal of the classical pattern. In colonies of the bee *Halictus ligatus* (Halictidae), queens produced most of the male-destined eggs whereas workers produced many of the gyne-destined eggs. We suggest that this pattern may result from temporal constraints on the production of reproductive brood, and that it may be common among primitively eusocial species.

The social behaviour of *Halictus ligatus* varies geographically^{9,13}. At our study site in southern Ontario, Canada, each overwintered foundress rears a brood consisting mainly of workers, with a few males. The workers then rear a second brood of males and gynes^{12,13}. Dissections showed that many workers had developed ovaries and filled spermathecae (Table 1). To test the implication that workers might be producing gynes, we assayed allozyme genotypes of nestmates at various times during the season. First-brood genotype arrays showed that some queens had mated more than once. Surprisingly, second-brood genotype arrays and relatedness estimates showed that in many nests two or more females had produced gynes (Table 2).

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TABLE 1 Matedness and ovarian development in *H. ligatus* workers collected from 28 nests with queens and developing reproductive brood in 1991

Mated	Ovarian development N (%)		
	Yes	No	Total
Yes	15 (35.7)	7 (16.7)	22 (52.4)
No	9 (21.4)	11 (26.2)	20 (47.6)
Total	24 (57.1)	18 (42.9)	42 (100.0)

Workers were classified as showing ovarian development if they had at least one developing oocyte that had grown to at least a quarter of its full size^{12,13}.

The average relatedness among workers in single-foundress nests is roughly 0.5, which implies that a queen typically uses equal amounts of sperm from two unrelated males. Worker-gyne relatedness is lower ($t = 5.3$, d.f. = 50, $P < 0.001$), and gyne-gyne relatedness is lower still ($t = 5.8$, d.f. = 59, $P < 0.001$), implying that the gynes in a nest typically have two or more female parents. Other interpretations can be rejected: queens rarely emerge from the nest after provisioning worker brood, so are unlikely to acquire additional mates before production of the second (reproductive) brood; and intraspecific nest parasitism was never seen during hundreds of hours of close observation¹³. Low worker-gyne and gyne-gyne relatedness (relative to worker-worker relatedness) therefore implies worker oviposition. In addition, queen-gyne relatedness is lower than expected for a mother-daughter relationship. Thus queens appear to produce most of the males and some gynes, whereas workers produce a few males and many (perhaps most) of the gynes.

Sex ratios provide additional evidence of 'inverted parentage'. First-brood males mate mainly with workers¹³, so they contribute to their mothers' fitness to the extent that workers produce gynes. If a colony's second-brood productivity is proportional to its number of workers, then a foundress's evolutionary equilibrium first-brood allocation to males is $m_1^* = \frac{1}{2}g$ (with $1 - m_1^*$ allocated to workers), where g is the population-wide proportion of gynes that are worker-produced. On average, the observed $m_1 \approx 0.15$, implying $g \approx 0.3$. If foundresses produce all the second-brood males, and if they are typically twice-mated, then a non-laying worker's second-brood equilibrium is $m_2^* = \frac{1}{2}(\frac{2}{3} + \frac{1}{3}g)$ (with $1 - m_2^*$ allocated to gynes). The observed $m_2 \approx 0.45$, implying that $g \approx 0.7$. Values of g in this range (0.3–0.7) are consistent

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TABLE 2 Estimated regression coefficients of relatedness (*R*) for various categories of nestmates from single-foundress nests of *H. ligatus*

Relationship	Expected <i>R</i>		1990 estimates		1991 estimates		Pooled: <i>R</i> (s.e.) [U.C.L.]
	H1	H2	<i>R</i> (s.e.) [U.C.L.]	<i>N_c</i> (<i>N_i</i>)	<i>R</i> (s.e.) [U.C.L.]	<i>N_c</i> (<i>N_i</i>)	
Queen-worker	0.5	0.5	0.55 (0.13) [0.79]	11(11,35)	0.38 (0.15) [0.66]	9(9,41)	0.47 (0.10) [0.64]
Queen-male	1.0	1.0	0.87 (0.13) [1.13]	7(7,18)	1.00 (0.00)	5(5,18)	0.91 (0.09) [1.08]
Queen-gyne	0.5	0.5	0.45 (0.24) [1.14]	3(3,15)	-0.18 (0.19) [0.27*†]	4(4,15)	0.11 (0.19) [0.49*†]
Worker-worker	0.75	0.5	0.32 (0.16) [0.61*]	12(45)	0.49 (0.11) [0.67*]	15(61)	0.42 (0.09) [0.57*]
Worker-male	0.5	0.5	0.31 (0.22) [0.72]	10(22,20)	0.40 (0.18) [0.70]	21(42,101)	0.37 (0.14) [0.60]
Worker-gyne	0.75	0.5	0.40 (0.23) [0.90]	5(9,16)	0.23 (0.13) [0.45*†]	20(42,111)	0.26 (0.11) [0.45*†]
Gyne-gyne	0.75	0.5	0.34 (0.14) [0.60*]	8(33)	0.28 (0.08) [0.41*†]	28(168)	0.29 (0.07) [0.41*†]

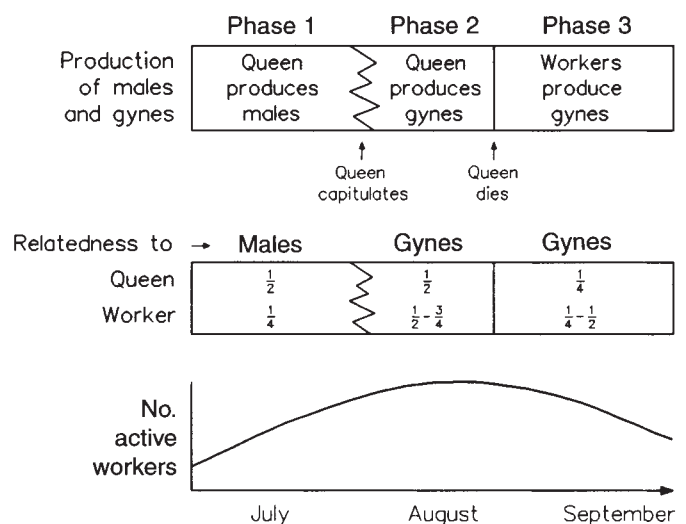
The single-foundress status of nests was confirmed by behavioural observation of marked foundresses and by size differences between foundresses and workers¹³. Expected coefficients of relatedness are based on the hypothesis that queens monopolize oviposition, whether singly mated (hypothesis 1, H1) or doubly mated, using sperm from both mates equally (hypothesis 2, H2). Genotypes for 549 individuals from 64 colonies were determined for Hk (hexokinase), a monomeric enzyme, and GDA (guanine deaminase), a dimeric enzyme; each had five alleles. Electrophoretic methods were slightly modified from ref. 28; additional details are given in ref. 13. The female-male regression coefficients (*R*) as calculated here by the method of Queller and Goodnight²⁹ are twice as large as the life-for-life coefficients²⁶ (*r*) used in applications of Hamilton's rule; for example, the life-for-life mother-son relatedness is $\frac{1}{2}$ and the sister-brother relatedness is $\frac{1}{4}$. Estimated standard errors are derived by jackknifing over colonies. Numbers of colonies (*N_c*) and individuals (*N_i*) are shown for 1990 and 1991; pooled samples are the sums of these. For relationships between the members of different castes, two sample sizes are given. The small sample sizes for 1990 reflect poor nest and brood survival that year. Coefficients involving queens are less reliable than those involving workers or gynes because of small sample sizes and sampling biases (only long-lived queens were found), but these coefficients are less critical for testing the hypothesis that many gynes derive from worker-laid eggs. One-sided 95% upper confidence limits [U.C.L.] for the estimated values of *R* are used to ask whether *R* is significantly smaller than the value expected under H1 (*) and the more conservative H2 (†). Rejection of H2 implies that even given multiple mating by the original foundress, the observed relatedness is lower than expected were she to monopolize oviposition.

with the relatedness estimates (Table 2); a mean slightly below $\frac{1}{2}$ seems most plausible, but there is clearly much colony-to-colony and year-to-year variation.

Reproductive-brood parentage may be strongly affected by midsummer weather. In 1990, when rain caused high rates of nest failure and queens produced relatively few, small workers,

queens may have been more successful in preventing worker oviposition than in the dry summer of 1991 when they produced more, larger workers¹³. Queen-gyne, worker-gyne and gyne-gyne relatedness estimates are all higher for 1990 than 1991 (Table 2). Thus bad weather in 1990 seems to have allowed queens to 'skew' reproduction^{14,15} more strongly in their favour

FIG. 1 Model for the production of males and gynes in colonies of *Halictus ligatus* and other primitively social bees with similar life histories. Nests are established in late spring by mated, overwintered gynes ('queens') who rear first (early-summer) broods consisting mainly of females ('workers') and a few males. After provisioning the last of her first-brood offspring, the queen ceases foraging and waits for adult workers to emerge. We propose that the second (late-summer) brood is typically produced in three phases, defined by the predominant source and sex of eggs. **Phase 1:** Workers emerge and begin foraging for pollen and nectar to provision second-brood offspring (males and gynes). The queen remains in the nest, directing the construction of brood cells and laying all the eggs. She produces many more males than females because early-emerging males have more mating opportunities than late-emerging males²⁰, and because the final population-wide investment ratio for the second brood will be female biased; the queen therefore tends to realize more inclusive fitness per unit invested in sons than in daughters, especially at this point in the season. **Phase 2:** Workers are less related to their brothers ($r = \frac{1}{4}$) than to their sisters ($r = \frac{3}{4}$ if the queen mated once, $r = \frac{1}{2}$ if she mated twice). Unless the final ratio of investment is very strongly female biased (between 2:1 and 3:1, depending on *r*), workers would gain more from rearing sisters than brothers¹⁻³. In phase 2 the workers gain control of the colony's sex allocation and switch it from predominantly male production to predominantly female production. They are able to do this because (1) as more workers emerge they increasingly outnumber the queen, (2) the nest increases in size and architectural complexity, such that the queen cannot patrol it as effectively as she could during phase 1, and (3) the queen becomes physically weaker as she ages. Confronted with large gyne-size brood cells and provision masses, and with the threat that workers might replace her male eggs with male or female eggs of their own ($r = \frac{1}{4} - \frac{3}{8}$ to non-laying workers, and $r = \frac{1}{2}$ to the egg layer), the queen 'capitulates' to worker 'demand' for fertilized, gyne-destined eggs (see refs 14, 15). **Phase 3:** The queen may live to the end of the season and lay most or all of the male- and gyne-destined



eggs in some nests, but in most nests she dies well before the end of the season^{12,13}. These nests become semisocial, with one or more mated workers taking over the role of principal egg layer(s). The new egg layers and their non-laying sisters 'agree' that most or all subsequent brood should be female, even though the overall investment ratio will be female biased, because males produced during phase 3 will have relatively few opportunities to mate. Relatedness estimates for *H. ligatus* at our study site near Victoria, Ontario (Table 2) suggest that at least in this population, phase 3 is often longer than phase 2, which may even be of zero length in some nests.

(by physically dominating fewer, smaller workers¹⁶), whereas good weather in 1991 allowed workers to obtain larger shares of their colonies' reproduction. Geographic variation in the structure of *H. ligatus* colonies may arise from similar causes; there is generally less reproductive skew at lower latitudes where the foraging season is long and colonies become relatively large⁹⁻¹³. Geographic variation has been documented in a few other halictids, but in these species harsher conditions (e.g. higher elevations) lead to the abandonment of sociality^{17,18}, an option that may be closed to *H. ligatus*.

There are other temperate halictids in which workers often mate¹⁹, foundresses rarely survive to the end of the summer, and males and gynes are produced sequentially, emerging over a period of several weeks near the end of the season. Late-emerging males have few mating opportunities in such species, so a queen's best strategy should be to produce male-destined eggs first²⁰⁻²³, then switch to gyne production when she loses control of her workers' behaviour (Fig. 1). If she subsequently dies, then her daughters will continue to produce females (their daughters and nieces), because the reproductive value of males produced late in the season will be very low. Thus, among primitively social species, the length of the period of emergence of males and gynes should be correlated with the extent of worker parentage of gynes. For example, *Lasioglossum laevisimum* has a relatively brief emergence period, and genetic evidence shows that it conforms to the classical eusocial pattern in which queens monopolize egg production⁵. Sensitive tests of the inverted-parentage model could be based on precise determinations of parentage in individual nests (using polymorphic microsatellite loci²⁴), in primitively social species with appropriate colony cycles.

Where the reproductive values of males and females depend differently on when they are produced, neither a worker nor a queen is indifferent to her colony's sex allocation, even if the local population is (from her point of view) in sex-ratio equilibrium^{20,23}. Time may then constrain the effects of factors that would otherwise increase sex-ratio heterogeneity among colonies. For example, orphaned (queenless) nests of the halictid *Augochlorella striata* produce sex ratios that are more male-biased than those of eusocial nests⁷, but this difference is modest compared both to what is seen in many ants with synchronized nuptial flights²⁵, and to what is predicted by models that treat investment as an instantaneous process^{26,27}. To the extent that the model described here succeeds in explaining how the period of emergence of males and gynes affects colony dynamics, it will focus attention on several poorly understood issues including variation in the period of emergence, the evolution of queen longevity, and transitions between annual and perennial colony cycles. □

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Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products

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FEMALE *Drosophila melanogaster* with environmentally¹⁻³ or genetically⁴ elevated rates of mating die younger than controls. This cost of mating is not attributable to receipt of sperm⁵. We demonstrate here that seminal fluid products from the main cells of the male accessory gland are responsible for the cost of mating in females, and that increasing exposure to these products increases female death rate. Main-cell products are also involved in elevating the rate of female egg-laying, in reducing female receptivity to further matings and in removing or destroying sperm of previous mates⁶⁻¹². The cost of mating to females may therefore represent a side-effect of evolutionary conflict between males¹³.

The proteins in the seminal fluid of male *D. melanogaster* are produced in the main and secondary cells of the male accessory gland and in the ejaculatory bulb and duct^{9,10,14-20}. The aim of our experimental design was to vary female exposure to main-cell products at mating, while keeping constant other costly aspects of reproduction such as egg-production²¹, non-mating exposure to males² and rate of mating. Females require both seminal fluid and sperm to initiate and maintain normal receptivity and rates of egg-production^{1,7,8,12}. All females were therefore intermittently exposed (1 day in 3) to intact wild-type males^{1,5}. Non-mating exposure to males was standardized by the use of control groups of males with their external genitalia microcauterized; these males behave normally but cannot mate^{1,5}. The females were exposed to either experimental, intact males or control, microcauterized males on the 2 days between each exposure to normal males.

In the initial experiment, two types of experimental (and control) males were used. The first were from a transgenic stock¹² carrying a construct in which the coding sequences for diphtheria toxin subunit A (DTA) were downstream of a main-cell promoter. DTA in the main cells of males of this stock kills the cells by blocking protein translation. These DTA males produce no detectable main-cell products in their seminal fluid and they also lack sperm but produce secondary cell and ejaculatory duct and bulb products¹². The second set of males ('tudor' males) were sons of tudor females, and produced normal seminal fluid but lacked germ-line cells and hence sperm^{12,22}. Microcauterized

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