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Partial ovary development is widespread in honey bees and comparable to other eusocial bees and wasps

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Honey bee workers have few opportunities for direct reproduction because their ovary development is chemically suppressed by queens and worker-laid eggs are destroyed by workers. While workers with fully developed ovaries are rare in honey bee colonies, we show that partial ovary development is common. Across nine studies, an average of 6% to 43% of workers had partially developed ovaries in queenright colonies with naturally mated queens. This shift by workers toward potential future reproduction is linked to lower productivity, which suggests that even small investments in reproductive physiology by selfish workers reduce cooperation below a theoretical maximum. Furthermore, comparisons across 26 species of bees and wasps revealed that the level of partial ovary development in honey bees is similar to that of other eusocial Hymenoptera where there is reproductive conflict among colony members. Natural variation in the extent of partial ovary development in honey bee colonies calls for an exploration of the genetic and ecological factors that modulate shifts in cooperation within animal societies.

Honey bees meet all three traditional criteria for eusociality[1,2] and are considered to be at the extreme of the eusociality continuum.[3,4] One reason for this view is that they exhibit high reproductive inequity (skew) in favor of the queen, in part because queens secrete chemicals that inhibit the development of workers' ovaries.[5,6] Because reproduction in a queenright honey bee colony is funneled almost exclusively through the queen, the genetic interests of colony members overlap greatly and the colony as a whole is expected to function as a highly cooperative, integrated and relatively harmonious "superorganism,"[7,8] where individuals partition their energy in favor of between-group competition (i.e., within-group cooperation) rather than within-group competition.[9]

However, inhibition of workers' ovary development is not perfect, providing opportunities for movement along a "superorganism continuum"[9] as reproductive conflict increases within colonies. Honey bee workers, who are unable to mate but are capable of producing eggs that give rise to haploid sons, produce ~7% of male eggs that are in colonies.[10] About 1 out of every 10,000 workers has fully developed eggs in their ovaries,[11] so only a small proportion of workers lay these eggs and most eggs are removed by other workers after they are laid.[11,12] Consequently, workers produce a vanishingly small percentage of adult males, about 0.12% per colony, giving workers very low rates of direct reproduction in queenright colonies.[12] Workers with fully developed ovaries are rarely observed outside of colonies that are queenless[13] or have genetic mutations that give workers unusually high rates of reproduction in the presence a queen.[14,15] Yet, there is an intriguing phenomenon that is often overshadowed by the starkly low numbers of workers with fully developed ovaries in queenright honey bee colonies—while full ovarian development in worker honey bees is rare, partial ovarian development is common.

Workers with partially developed ovaries have ovaries that are neither resting (i.e., no swelling of the ovarioles) nor at an advanced stage of development (i.e., completely elongated eggs visible within ovarioles)[16] [based on Velthuis (with photographs)].[17] Across several studies, the occurrence of workers with partially developed ovaries in queenright colonies is widespread, ranging from a mean of 6–43% of worker populations (**Table 1**). When compiling these data, we only considered colonies that were headed by naturally mated queens that were not subject to experimental manipulation (i.e., controls). There was substantial variation across these studies in the proportion of workers that had partially developed ovaries, which is probably attributable to differences in year, location, season, genetics and dissection methodology.[18,19] Nevertheless, it is clear that partial ovary development is consistently observed among workers in queenright colonies. This observation raises the question: if workers with only partially developed ovaries are effectively sterile, what is the significance of this incomplete investment in reproductive physiology? In particular, what implications does it have for our understanding of the extent of cooperation within honey bee

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Table 1. The occurrence of partial ovary development among workers in honey bee colonies

Study	Scale	Mean % (± s.e.m.)	% Range	No. colonies (no. individuals)	Source of data
Amdam et al. (2006)[30]	1, **2, 3,** 4	18±11	0–46%	4 (120)	Queenright colony pairs #5 and #6 (in Supplemental Material)
Backx et al. (2012)[18]	0, **1, 2**, 3, 4	43±5	25–55%	5 (638)	Untreated queenright colonies (data provided by authors)
Jay (1970)[31]	0, **I,** II	6±1	4–9%	4 (300)	Queenright controls (from Tables 1 and 2)
Kropacova and Haslbachova (1969)[32]	I, **II, III**, IV, V	17±1	14–21%	6 (3,540)	Non-swarming queenright colonies (from Figure 1)
Kropacova and Haslbachova (1970)[33]	I, **II, III**, IV, V	37	n/a	7 (390)	Pre-swarming queenright colonies (from Figure 1)
Kropacova and Haslbachova (1971)[34]	I, **II, III**, IV, V	31	n/a	3 (2,700)	Group IV: unmanipulated control (from Figures 1–3)
Makert et al. (2006)[35]	A, **B,** C	13±5	8–18%	2 (137)	Queenright colonies A and B (from Table 2)
Mattila et al. (2012)[21]	0, **1, 2, 3**, 4	22±3	0–50%	14 (521)	Naturally mated queens in 2008; queenright halves in 2010
Woyciechowski and Kuszewska (2012)[29]	1, **2, 3**, 4	27± 8	0–57%	6 (180)	Queenright colonies #1–3, #7–9 (in Supplemental Material)

Data were collated from multiple studies; values are provided for colonies in those studies only if they were queenright, had naturally mated queens, and had not undergone an experimental manipulation (i.e., they were control colonies; see notes in far right column). Cited authors used a variety of scales to score ovary development (second column); we considered scores in bold to fall into the category of partial ovary development, according to Pernal and Currie (2000) and based on images in Velthuis (1970).[17] Authors combined colonies when reporting ovary development, so range and s.e.m. were not available.

colonies and our perception of them as superorganisms with closely aligned genetic interests?

The reasons why substantial numbers of honey bee workers have partially developed ovaries remain unclear. While the potential costs to colonies of unrestrained reproduction by workers are avoided in honey bees,[20] even small increases in the proportion of workers with partial ovary development are correlated with meaningful decreases in colony productivity, specifically colony-wide rates of foraging and recruitment (Figure 2 in ref. 20). Even if the purpose for partial ovary development is to become reproductively ready for possible queen loss,[12,21] and not to reproduce in the presence of the queen when worker-worker relatedness favors it,[22] this selfish, albeit limited, investment by workers in their own reproductive potential still carries productivity costs for colonies. While highly effective policing may reduce conflict in honey bee colonies by making cooperation, and not selfish reproduction, the best option for honey bee workers,[23] substantial levels of partial ovary development probably signal some level of intracolony conflict. Bet-hedging workers may be pushing ovary development only to the point where they can continue to avoid detection as reproductive cheaters[24] and the social sanctions (i.e., aggression) that come with it.[25] If so, then these limited investments by selfish workers in possible future opportunities for reproduction may reduce cooperation among colony members below a theoretical maximum.

How does the phenomenon of partial ovary development in honey bees fit into a broader perspective of ovary development in the eusocial Hymenoptera? **Table 2** provides data on the degree of ovary development for wasps and bees other than *Apis mellifera*. The table is not exhaustive and interspecies comparisons are complicated by differences in life histories, colony structure and degree of reproductive conflict among colony members. For example, many stingless bees produce trophic eggs that are used to feed queens, so it is difficult to discriminate the development of ovaries for laying trophic eggs from the production of reproductive eggs.[26] However, we can make some general statements across eusocial taxa regarding investment by workers in ovarian development. At first glance, it is clear that honey bees have very low proportions of workers with fully developed ovaries compared with other species (**Table 2**). However, the level of partial ovary development in *A. mellifera* is comparable to other *Apis* species, stingless bees and wasps. If partial ovary development in honey bee workers reflects the first stages of becoming reproductive, then substantial reproductive conflict persists among honey bees, even though they have been typically viewed as having some of the strictest sanctions against worker reproduction,[23] sanctions that are apparently much stronger than in their closest eusocial relatives, the stingless bees.[27] The widespread occurrence of partial ovary development indicates that a honey bee colony falls significantly short of being the "perfect" superorganism and, instead, it hints at costly, actual reproductive conflict (sensu Reeve and Ratnieks) among its ranks.[28] Importantly, natural variation in the frequency of partly activated ovaries in honey bee colonies (**Table 1**) that fluctuate with shifting colony

Table 2. Comparison of ovary development for workers of multiple species of bees and wasps (Order Hymenoptera)

Family	Study	Species	No. colonies (no. individuals)	% Ovary development			Notes about source of data
				Resting	Partial	Fully	
Apidae (Tribe Apini)	Mattila et al. (2012)[21]	*Apis mellifera*	11 (220)	83%	15%	2%	Naturally mated queens in 2008
	Wattanachai-yingcharoen et al. (2006)[36]	*Apis dorsata*	8 (1,902)	66%	34%	0%	Partial = visible ovarioles (from Table 2)
	Oldroyd et al. (2001)[37]	*Apis cerana*	4 (800)	24%	71%	5%	Resting = ovarioles not discernible; partial = ovarioles visible; fully = eggs < 50% full sized (from Table 1)
Apidae (Tribe Meliponini)	Sakagami et al. (1963)[38]	*Lestrimelitta ehrhardti*	1 (14)	64%	36%	0%	Resting = A + F partial = B + C + E; fully = D; data combined for bees in stage II (nurses) and III (guards) (from Table 1)
		Trigona spinipes	1 (20)	20%	30%	50%	
		Trigona freiremaiai	1 (31)	48%	52%	0%	
		Trigona jaty	1 (21)	19%	38%	43%	
		Plebeia droryana	1 (20)	50%	35%	15%	
		Partamona schrottkyi	1 (21)	52%	19%	29%	
		Partamona cupira	1 (30)	90%	7%	3%	
		Nannotrigona testaceicornis	1 (30)	73%	10%	17%	
		Nannotrigona postica	1 (30)	63%	7%	30%	
		Nannotrigona xanthothryca	1 (21)	4%	48%	48%	
		Nannotrigona bipunctata	1 (20)	20%	30%	50%	
		Meliponula bocandei	1 (24)	42%	50%	8%	
		Melipona quadrifasciata	1 (10)	0%	20%	80%	
		Melipona marginata	1 (22)	23%	27%	50%	
Vespidae (Tribe Epiponini)	Felippotti et al. (2010)[39]	*Clypearia sulcata*	3 (98)	54%	25%	21%	Resting = A; partial = B; fully = C + D (from Table 1)
		Clypearia angustior	1 (14)	45%	33%	22%	
		Clypearia duckei	1 (119)	34%	12%	54%	
	Gelin et al. (2008)[40]	*Angiopolybia pallens*	9 (549)	42%	20%	38%	Resting = A; partial = B; fully = C (from Table 1)
	Mateus et al. (2004)[41]	*Parachartergus fraternus*	5 (1,153)	65%	3%	32%	Resting = pattern 1; partial = pattern 2 + 3; fully = pattern 4 + 5 (from Table 1)
Vespidae (subfamily Vespinae)	Ross (1984)[42]	*Paravespula flavopilosa*	1 (40)	95%	5%	0%	Resting = 1; partial = 2; fully = 3 + 4 (% estimated from Figure 2)
		Paravespula germanica	1 (40)	85%	10%	5%	
		Paravespula maculifrons	1 (40)	79%	12%	9%	
		Paravespula vulgaris	1 (40)	85%	7%	8%	

Data reported are for queenright colonies with naturally mated queens (see notes in far right column).

conditions[21,29] opens the door to the study of genetic and ecological factors that modulate the degree of intracolony cooperation in animal societies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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