

5-21-2013

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

Michael L. Smith

Heather R. Mattila
hmattila@wellesley.edu

Follow this and additional works at: <http://repository.wellesley.edu/scholarship>

Version: Publisher's version

Recommended Citation

Michael L. Smith, Heather R. Mattila & H. Kern Reeve (2013) Partial ovary development is widespread in honey bees and comparable to other eusocial bees and wasps, *Communicative & Integrative Biology*, 6:5, e25004, DOI: 10.4161/cib.25004

This Article is brought to you for free and open access by Wellesley College Digital Scholarship and Archive. It has been accepted for inclusion in Faculty Research and Scholarship by an authorized administrator of Wellesley College Digital Scholarship and Archive. For more information, please contact ir@wellesley.edu.

This article was downloaded by: [Wellesley College]

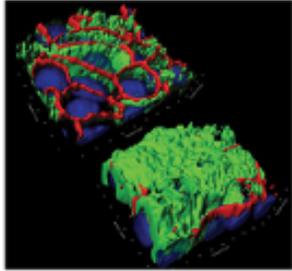
On: 17 March 2015, At: 12:16

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Communicative
& Integrative **BIOLOGY**

Volume 6 • Issue 5 • September/October 2013



Communicative & Integrative Biology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/kcib20>

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

Michael L. Smith^a, Heather R. Mattila^b & H. Kern Reeve^a

^a Department of Neurobiology and Behavior; Cornell University; Ithaca, NY USA

^b Department of Biological Sciences; Wellesley College; Wellesley, MA USA

Published online: 21 May 2013.

To cite this article: Michael L. Smith, Heather R. Mattila & H. Kern Reeve (2013) Partial ovary development is widespread in honey bees and comparable to other eusocial bees and wasps, *Communicative & Integrative Biology*, 6:5, e25004, DOI: [10.4161/cib.25004](https://doi.org/10.4161/cib.25004)

To link to this article: <http://dx.doi.org/10.4161/cib.25004>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Versions of published Taylor & Francis and Routledge Open articles and Taylor & Francis and Routledge Open Select articles posted to institutional or subject repositories or any other third-party website are without warranty from Taylor & Francis of any kind, either expressed or implied, including, but not limited to, warranties of merchantability, fitness for a particular purpose, or non-infringement. Any opinions and views expressed in this article are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor & Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

It is essential that you check the license status of any given Open and Open Select article to confirm conditions of access and use.

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

Michael L. Smith,^{1,*} Heather R. Mattila² and H. Kern Reeve¹

¹Department of Neurobiology and Behavior; Cornell University; Ithaca, NY USA; ²Department of Biological Sciences; Wellesley College; Wellesley, MA USA

Keywords: ovary development, partial ovary development, honey bees, eusocial bees, wasps, reproductive conflict, intra-colony cooperation, reproductive physiology

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 queenless¹³ 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

*Correspondence to: Michael L. Smith; Email: mls453@cornell.edu

Submitted: 01/23/13; Revised: 05/08/13; Accepted: 05/08/13

Citation: Smith ML, Mattila HR, Reeve HK. Partial ovary development is widespread in honey bees and comparable to other eusocial bees and wasps. *Commun Integr Biol*; 6: e25004; <http://dx.doi.org/10.4161/cib.25004>

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 cheaters²⁴ 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	<i>Apis mellifera</i>	11 (220)	83%	15%	2%	Naturally mated queens in 2008	
	Wattanachaiyingcharoen et al. (2006) ³⁶	<i>Apis dorsata</i>	8 (1,902)	66%	34%	0%	Partial = visible ovarioles (from Table 2)	
	Oldroyd et al. (2001) ³⁷	<i>Apis cerana</i>	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	<i>Lestrimelitta ehrhardti</i>	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)	
		<i>Trigona spinipes</i>	1 (20)	20%	30%	50%		
		<i>Trigona freiremaiai</i>	1 (31)	48%	52%	0%		
		<i>Trigona jaty</i>	1 (21)	19%	38%	43%		
		<i>Plebeia droryana</i>	1 (20)	50%	35%	15%		
		<i>Partamona schrottkyi</i>	1 (21)	52%	19%	29%		
		<i>Partamona cupira</i>	1 (30)	90%	7%	3%		
		<i>Nannotrigona testaceicornis</i>	1 (30)	73%	10%	17%		
		<i>Nannotrigona postica</i>	1 (30)	63%	7%	30%		
		<i>Nannotrigona xanthothryca</i>	1 (21)	4%	48%	48%		
		<i>Nannotrigona bipunctata</i>	1 (20)	20%	30%	50%		
Vespidae (Tribe Epiponini)	Felippotti et al. (2010) ³⁹	<i>Clypearia sulcata</i>	3 (98)	54%	25%	21%	Resting = A; partial = B; fully = C + D (from Table 1)	
		<i>Clypearia angustior</i>	1 (14)	45%	33%	22%		
		<i>Clypearia duckei</i>	1 (119)	34%	12%	54%		
	Gelin et al. (2008) ⁴⁰	<i>Angiopolybia pallens</i>	9 (549)	42%	20%	38%	Resting = A; partial = B; fully = C (from Table 1)	
	Mateus et al. (2004) ⁴¹	<i>Parachartergus fraternus</i>	5 (1,153)	65%	3%	32%	Resting = pattern 1; partial = pattern 2 + 3; fully = pattern 4 + 5 (from Table 1)	
	Vespidae (sub-family Vespinae)	Ross (1984) ⁴²	<i>Paravespula flavopilosa</i>	1 (40)	95%	5%	0%	Resting = 1; partial = 2; fully = 3 + 4 (% estimated from Figure 2)
			<i>Paravespula germanica</i>	1 (40)	85%	10%	5%	
<i>Paravespula maculifrons</i>			1 (40)	79%	12%	9%		
<i>Paravespula vulgaris</i>			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.

Acknowledgments

Many thanks to G. Thompson for providing additional data and to K. Loope for providing comments on the manuscript. This material is based on work supported by a US National Science Foundation Graduate Research Fellowship (to M.L.S.) and a Knafel Endowed Chair in the Natural Sciences (to H.R.M.).

References

- Batra SW. Social behavior and nests of some nomiid bees in India (Hymenoptera, Halictidae). *Insectes Soc* 1966; 13:145-53; <http://dx.doi.org/10.1007/BF02223020>
- Wilson EO. *The Insect Societies*. (Belknap Press: 1971).
- Lacey E, Sherman P. Redefining eusociality: concepts, goals and levels of analysis. *Ann Zool Fenn* 2005; 573-7
- Sherman P, Lacey E, Reeve H, Keller L. Forum: The eusociality continuum. *Behav Ecol* 1995; 6:102-8; <http://dx.doi.org/10.1093/beheco/6.1.102>
- Hoover SER, Keeling CI, Winston ML, Slessor KN. The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* 2003; 90:477-80; PMID:14564409; <http://dx.doi.org/10.1007/s00114-003-0462-z>
- Voogd S. The influence of a queen on the ovary development in worker bees. *Cell Mol Life Sci* 1956; <http://dx.doi.org/10.1007/BF02170795>
- Seeley T. The honey bee colony as a superorganism. *Am Sci* 1989; 77:546-53
- Hölldobler B, Wilson EO. *The superorganism: the beauty, elegance, and strangeness of insect societies*. (W.W. Norton & Company: 2009).
- Reeve HK, Hölldobler B. The emergence of a superorganism through intergroup competition. *Proc Natl Acad Sci USA* 2007; 104:9736-40; PMID:17517608; <http://dx.doi.org/10.1073/pnas.0703466104>
- Visscher PK. Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing. *Behav Ecol Sociobiol* 1996; 39:237-44; <http://dx.doi.org/10.1007/s002650050286>
- Ratnieks FW. Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. *Behav Ecol Sociobiol* 1993; 32:191-8; <http://dx.doi.org/10.1007/BF00173777>
- Visscher P. A quantitative study of worker reproduction in honey bee colonies. *Behav Ecol Sociobiol* 1989; 25:247-54; <http://dx.doi.org/10.1007/BF00300050>
- Page RE, Robinson GE. Reproductive competition in queenless honey bee colonies (*Apis mellifera* L.). *Behav Ecol Sociobiol* 1994; 35:99-107; <http://dx.doi.org/10.1007/BF00171499>
- Barron A, Oldroyd B, Ratnieks F. Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: a review. *Behav Ecol Sociobiol* 2001; 50:199-208; <http://dx.doi.org/10.1007/s002650100362>
- Neumann P, Moritz R. The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? *Behav Ecol Sociobiol* 2002; 52:271-81; <http://dx.doi.org/10.1007/s00265-002-0518-7>
- Pernal S, Currie R. Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). *Apidologie (Celle)* 2000; 31:387-409; <http://dx.doi.org/10.1051/apido:2000130>
- Velthuis H. Ovarian development in *Apis mellifera* worker bees. *Entomol Exp Appl* 1970; 13
- Backx AG, Guzmán-Novoa E, Thompson GJ. Factors affecting ovary activation in honey bee workers: a meta-analysis. *Insectes Soc* 2012; 59:381-8; <http://dx.doi.org/10.1007/s00040-012-0230-1>
- Hoover SER, Higo HA, Winston ML. Worker honey bee ovary development: seasonal variation and the influence of larval and adult nutrition. *J Comp Physiol B* 2006; 176:55-63; PMID:16228242; <http://dx.doi.org/10.1007/s00360-005-0032-0>
- Nonacs P. Nepotism and brood reliability in the suppression of worker reproduction in the eusocial Hymenoptera. *Biol Lett* 2006; 2:577-9; PMID:17148292; <http://dx.doi.org/10.1098/rsbl.2006.0549>
- Mattila HR, Reeve HK, Smith ML. Promiscuous honey bee queens increase colony productivity by suppressing worker selfishness. *Curr Biol* 2012; 22:2027-31; PMID:23022065; <http://dx.doi.org/10.1016/j.cub.2012.08.021>
- Loope KJ, Seeley TD, Mattila HR. No facultative worker policing in the honey bee (*Apis mellifera* L.). *Naturwissenschaften* 2013; 100:473-7; PMID:23455897; <http://dx.doi.org/10.1007/s00114-013-1025-6>
- Wenseleers T, Ratnieks F. Enforced altruism in insect societies. *Nature* 2006; 444:50-9; PMID:17080043; <http://dx.doi.org/10.1038/444050a>
- Smith AA, Hölldobler B, Liebig J. Cuticular hydrocarbons reliably identify cheaters and allow enforcement of altruism in a social insect. *Curr Biol* 2009; 19:78-81; PMID:19135369; <http://dx.doi.org/10.1016/j.cub.2008.11.059>
- Visscher PK, Dukas R. Honey bees recognize development of nestmates' ovaries. 1995: 542-44.
- Cepeda O. Division of labor during brood production in stingless bees with special reference to individual participation. *Apidologie (Celle)* 2006; 37:175-90; <http://dx.doi.org/10.1051/apido:2006018>
- Toth E, Queller DC, Dollin A, Strassmann JE. Conflict over male parentage in stingless bees. *Insectes Soc* 2004; 51:1-11; <http://dx.doi.org/10.1007/s00040-003-0707-z>
- Reeve HK, Ratnieks FLW. Queen-queen conflicts in polygynous societies: mutual tolerance and reproductive skew. *Queen number and sociality in insects* (1993).
- Woyciechowski M, Kuszewska K. Swarming generates rebel workers in honeybees. *Curr Biol* 2012; 22:707-11; PMID:22464193; <http://dx.doi.org/10.1016/j.cub.2012.02.063>
- Amdam GV, Csondes A, Fondrk MK, Page RE Jr. Complex social behaviour derived from maternal reproductive traits. *Nature* 2006; 439:76-8; PMID:16397498; <http://dx.doi.org/10.1038/nature04340>
- Jay S. The effect of various combinations of immature queen and worker bees on the ovary development of worker honeybees in colonies with and without queens. *Can J Zool* 1970; <http://dx.doi.org/10.1139/z70-019>
- Kropacova S, Haslbachova H. The development of ovaries in worker honeybees in a queenright colony. *J Apic Res* 1969
- Kropacova S, Haslbachova H. The development of ovaries in worker honeybees in queenright colonies examined before and after swarming. *J Apic Res* 1970
- Kropacova S, Haslbachova H. The influence of queenlessness and of unsealed brood on the development of ovaries in worker honeybees. *J Apic Res* 1971
- Makert GR, Paxton RJ, Hartfelder K. Ovariole number—a predictor of differential reproductive success among worker subfamilies in queenless honeybee (*Apis mellifera* L.) colonies. *Behav Ecol Sociobiol* 2006; 60:815-25; <http://dx.doi.org/10.1007/s00265-006-0225-x>
- Wattanachaiyingcharoen W, et al. Lack of worker reproduction in the giant honey bee *Apis dorsata* Fabricius. *Insectes Soc* 2006; 49:80-5; <http://dx.doi.org/10.1007/s00040-002-8283-1>
- Oldroyd B, et al. Worker policing and worker reproduction in *Apis cerana*. *Behav Ecol Sociobiol* 2001; 50:371-7; <http://dx.doi.org/10.1007/s002650100376>
- Sakagami S, Beig D, Zucchi R, Akahira Y. Occurrence of ovary-developed workers in queenright colonies of stingless bees. *Rev Bras Biol* 1963
- Felippotti GT, Mateus L, Mateus S, Noll FB, Zucchi R. Morphological caste differences in three species of the neotropical genus *Clypearia* (Hymenoptera: Polistinae: Epiponini). *Psyche (Camb, Mass)* 2010; <http://dx.doi.org/10.1155/2010/410280>
- Gelin LFF, Cruz JD, Noll FB, Giannotti E, Santos GM, Bichara-Filho CC. Morphological caste studies in the neotropical swarm-founding Polistinae wasp *Angiopolybia pallens* (Lepeletier) (Hymenoptera: Vespidae). *Neotrop Entomol* 2008; 37:691-701; PMID:19169558; <http://dx.doi.org/10.1590/S1519-566X2008000600011>
- Mateus S, Noll F, Zucchi R. Caste flexibility and variation according to the colony cycle in the swarm-founding wasp, *Parachartergus fraternus* (Gribodo) (Hymenoptera: Vespidae: Epiponini). *J Kans Entomol Soc* 2004; 77:470-83; <http://dx.doi.org/10.2317/E-40.1>
- Ross KG. Aspects of worker reproduction in four social wasp species (Insecta: Hymenoptera: Vespidae). *J Zool* 1984; 205:411-24; <http://dx.doi.org/10.1111/j.1469-7998.1985.tb05626.x>