

## ECOLOGY, BEHAVIOR AND BIONOMICS

# Castes in the Neotropical Social Wasp *Leipomeles dorsata* (Fabricius) (Hymenoptera: Vespidae): a Window for Workers Achieving a New Status in the Colony

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**ABSTRACT** - Morphometric studies performed in several species of Neotropical social wasps from the tribe Epiponini showed that in some species there are marked differences between castes, while other species present highly distinct castes with differences only in ovarian development. This work analyzed females from six colonies of the social wasp *Leipomeles dorsata* (Fabricius) in which queens (egglayers) and workers showed differences in ovarian development and coloration. We propose that wasps with developed ovaries (egglayers) and coloration similar to those of workers are possibly intermediates that obtained the status of queens in the colony.

**KEY WORDS:** Epiponini, caste difference, multivariate analysis

Theories for the evolution of eusociality (Hamilton 1964a,b, Alexander 1974) have greatly directed studies and discussions toward the potential contribution of females in the gene pool of the next generation. Worker sterility is now a paradigm (West-Eberhard 1975, Anderson 1984, Brockman 1984). However, it is often only partial, and workers often combine the usual helper repertory with some kind of reproduction-related activities (Bourke 1988). In highly eusocial insects, striking examples are found in stingless bees (Zucchi 1993) and Neotropical swarm-founding wasps (Noll *et al* 2004).

In social wasps (Vespidae), the Epiponini is a very intriguing neotropical tribe of Polistinae (Carpenter 2004). The whole tribe is highly eusocial and polygynic (i.e., several egglayers commonly reproduce simultaneously on the same nest). Their societies are complex (Bourke 1998) because all reproduce by swarms (Jeanne 1991) and division of labor is highly specialized (O'Donnell 1998). Castes in this tribe challenge standard definitions. As expected for highly eusocial insects, pre-imaginal caste determination has been reported in at least five genera (Noll *et al* 2004). However, in at least six genera (Noll *et al* 2004), reproductive females resemble non-reproductives in terms of morphology, and castes also lack physiological discontinuities. In these cases, castes are flexible and determined by disputes among adults rather than by larval manipulation (West-Eberhard 1981, Mateus *et al* 2004, Noll & Wenzel 2008).

*Leipomeles* is part of a clade that bears slight morphological

caste differences. *Leipomeles dorsata* (Fabricius) are very shy, and when disturbed, females hardly demonstrate aggressiveness (Richards 1978). According to Noll *et al* (2004), egglayers and workers of *Metapolybia* (Ducke) and *Synoeca* (L.) lack morphological differences during the colonial cycle and females are able to develop their ovaries only in some phases of the colonial cycle. In this paper, we analyzed in detail the morphology and physiology of castes in *L. dorsata* using several colonies and found that some workers may reach the egglayer status, leading reproduction in the absence of a regular egglayer.

### Material and Methods

Six colonies of *L. dorsata*, collected in Presidente Figueiredo, AM, Brazil (01° 49' 596'' S, 060° 12' 078'' W) were collected with plastic bags at nightfall or in early morning and all adult wasps from each colony were preserved in 95% ethanol immediately after collection.

Caste was determined by examination of the ovaries and insemination. In order to verify insemination, the spermatheca was removed and analyzed. The presence of sperm cells was detected under a microscope. Groups for analysis were defined based on ovarian development and insemination. egglayers were defined as inseminated females, intermediates as females with some ovarian development, and workers as females without ovarian development.

Intermediates and workers were unseminated females. However, it is important to point out that the definition of these groups is not an indication that they are castes or sub-castes. Hereafter, intermediates and workers will be treated simply as workers.

In order to estimate the relative age of adult wasps by noting the progressive pigmentation of the transverse apodeme across the hidden base of each sternum, all measured individuals were analyzed under a stereomicroscope. Females were classified as young (without or with incipient pigmentation) (Fig 1a), middle-aged (light pigmentation)

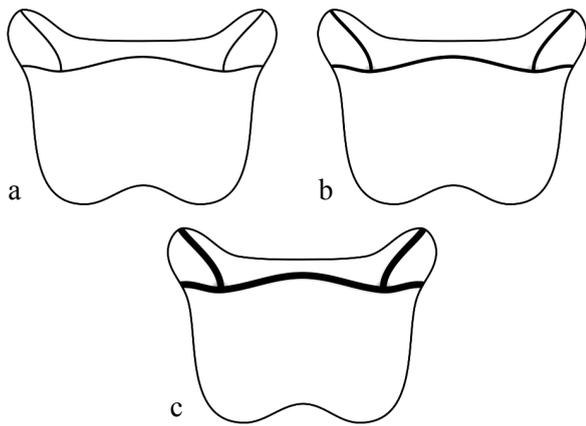


Fig 1 Age discrimination using the color of the transverse apodeme across the hidden base of each sternum. a) young female (without or with incipient pigmentation); b) middle-aged (light pigmentation); c) old (dark pigmentation).

(Fig 1b) and old (dark pigmentation) (Fig 1c) following Richards (1971) and West-Eberhard (1973).

All individuals of each colony were used for morphometric analysis. Measurements were taken from eight morphometric variables in each female (Fig 2). Measurements were taken using a stereomicroscope equipped with a video camera linked to a computer. Egglayer and worker differences for each variable were tested using the Bonferroni-corrected *t*-test after ANOVA analyses. A stepwise discriminant analysis was used to identify the most significant contributors for caste distinction. Later, the most discriminant characters were plotted for caste discrimination. Wilks' Lambda values were used to infer the individual contribution of each variable to the model. In order to check the efficiency of the test, a classification matrix test was used to check the number and percent of correctly classified cases in each group.

## Results

**Physiological differences and females' age.** Four patterns of ovarian development were identified in the studied colonies ranging from undeveloped to fully developed (Table 1): 1 – filamentous ovarioles bearing no visible oocytes or slightly developed oocytes, 2 – large immature oocytes located at the base of the ovary, 3 – well-developed oocytes and at least one mature oocyte, 4 – well-developed ovarioles with several mature oocytes. Patterns 1, 3 and 4 were found in all colonies, except colony 2 that lacked pattern 3 and colony 40 that lacked pattern 4. Pattern 2 was found only in colonies 27 and 31. Insemination was found only in females having

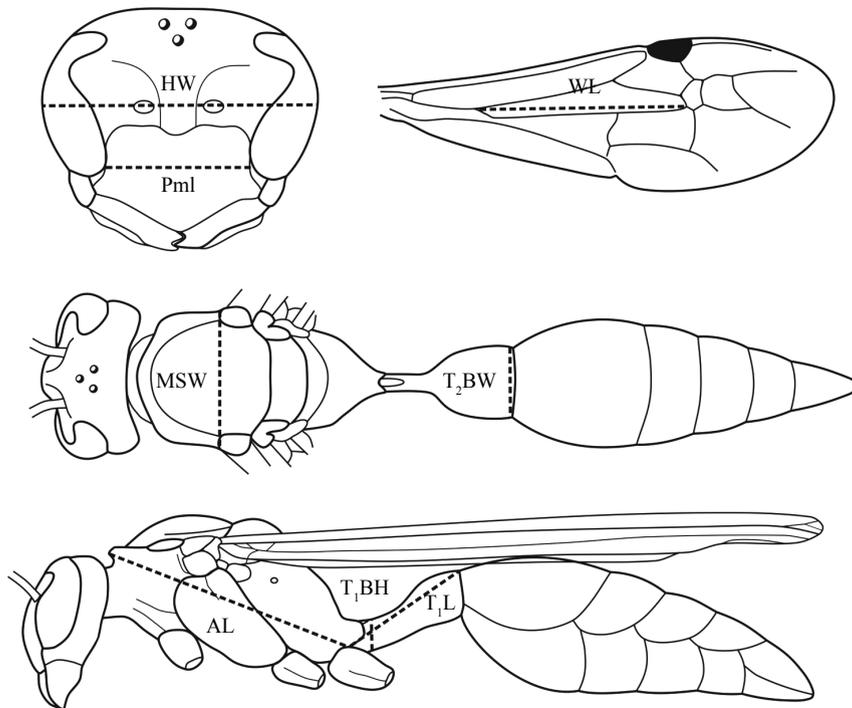


Fig 2 Representative measures for morphometric analyses of *Leipomeles dorsata* individuals: PML, minimum interorbital distance; HW, head width; MSW, mesoscutellar width; AL, alitrunk length; T<sub>1</sub>BH, basal width tergum I; T<sub>2</sub>BW, basal width tergum II; T<sub>1</sub>L, maximum width tergum I; WL, partial length of forewing (modified from Noll *et al* 2004).

Table 1 Patterns of ovarian development in six analyzed colonies of *Leipomeles dorsata*.

Colony code	Ovarian patterns (number of females)			
	1	2	3	4
2	93	-	-	4
25	63	-	7	5
26	71	-	2	13
27	77	3	7	1
31	49	3	9	2
40	30	-	2	-

Pattern 1 – filamentous ovarioles bearing no visible oocytes or slightly developed oocytes, Pattern 2 – large immature oocytes located in the base of the ovary, Pattern 3 – well-developed oocytes and at least one mature oocyte, Pattern 4 – well-developed oocytes with several mature oocytes.

patterns 3 and 4, and apparently, they are related to two color patterns found in egglayers (see below).

Females were classified in three different relative ages: young, middle-age and old (Fig 3). All colonies show individuals with different ages except in colony 27, that all egglayers and workers were old, and colony 40 all egglayers and workers were young individuals (Fig 3). The observed differences in the age of individuals certainly indicates different phases of the colonial cycle (Mateus *et al* 2004).

**Morphological differences.** Morphological studies indicated low distinction based on size (Table 2). Except in colonies 2 and 26, no statistical differences were found between egglayers and workers (Table 3). Egglayers from colony 2 were larger than workers, but the opposite was observed in colony 26 (Table 2). Multivariate analysis indicated that the differences found were very low (Tables 2 and 3) as observed by the high Wilks’ lambda values (Table 2), which ranged from 0.71 to

Table 2 Discriminant morphometric variables between queens and workers in *Leipomeles dorsatas* based on discriminant function analyses using the stepwise procedure.

Colony code	Variable	Wilks’ Lambda	F	P
2	HW	0.75	5.68	0.02
	T1BH	0.78	10.55	0.00
	MSW	0.74	4.67	0.03
	PML	0.73	3.86	0.05
	T1L	0.71	1.05	0.31
25	AL	0.99	3.26	0.08
	MSW	0.96	1.16	0.29
26	WL	0.85	6.73	0.01
	T1L	0.83	4.91	0.03
	MSW	0.80	1.70	0.20
	T2BW	0.80	1.13	0.29
27	T1L	0.96	2.18	0.14
	MSW	0.97	3.63	0.06
	AL	0.95	1.75	0.19
31	WL	0.92	4.14	0.05
	T1L	0.90	2.65	0.11
	T2BW	0.88	1.48	0.23
	PML	0.88	1.27	0.26
40	AL	0.88	4.23	0.05
	T1L	0.86	3.41	0.08
	T2BW	0.84	2.75	0.11

PML, minimum interorbital distance; HW, head width; MSW, mesoscutellar width; AL, alitrunk length; T1BH, basal width tergum I; T2BW, basal width tergum II; T1L, maximum width tergum I; WL, partial length of forewing. Original data are based on mm.

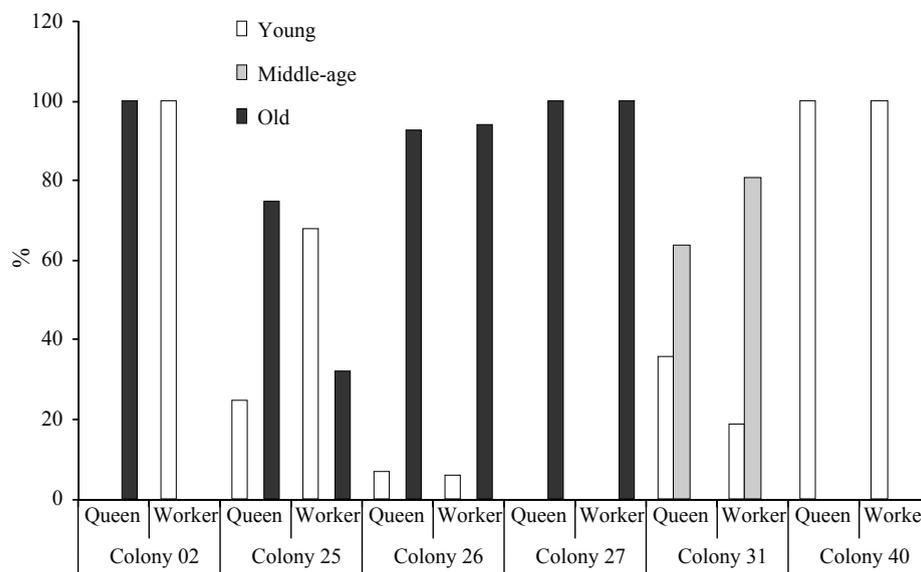


Fig 3 Relative age of egglayers and workers in the analyzed colonies of *Leipomeles dorsata*.

Table 3 Means and standard deviation (SD) and ANOVA test for females of *Leipomeles dorsata*. PML, minimum interorbital distance; HW, head width; MSW, mesoscutellar width; AL, alitrunk length; T1BH, basal width tergum I; T2BW, basal width tergum II; T1L, maximum width tergum I; WL, partial length of forewing.

Variables	Colony 2			Colony 25			Colony 26		
	Queens n = 04	Workers n = 93	ANOVA (F)	Queens n = 12	Workers n = 63	ANOVA (F)	Queens n = 15	Workers n = 71	ANOVA (F)
HW	1.93 ± 0.03	1.87 ± 0.03	17.68***	1.72 ± 0.04	1.72 ± 0.04	0.55	1.86 ± 0.04	1.89 ± 0.04	6.87**
PML	0.69 ± 0.01	0.70 ± 0.02	0.16	0.62 ± 0.02	0.62 ± 0.02	1.08	0.65 ± 0.02	0.66 ± 0.02	1.40
MSW	1.22 ± 0.03	1.14 ± 0.04	17.05***	1.15 ± 0.21	1.11 ± 0.15	0.83	1.13 ± 0.03	1.13 ± 0.04	0.68
T2BW	0.80 ± 0.04	0.77 ± 0.03	4.43*	0.70 ± 0.02	0.70 ± 0.03	0.01	0.79 ± 0.03	0.80 ± 0.04	2.20
AL	2.37 ± 0.05	2.30 ± 0.06	3.82*	2.04 ± 0.07	2.07 ± 0.07	2.95	2.23 ± 0.05	2.28 ± 0.07	7.64**
T1BH	0.20 ± 0.01	0.22 ± 0.02	3.11	0.20 ± 0.02	0.21 ± 0.03	0.52	0.24 ± 0.03	0.24 ± 0.02	0.06
T1L	1.59 ± 0.07	1.52 ± 0.05	8.40***	1.30 ± 0.05	1.31 ± 0.05	0.70	1.45 ± 0.04	1.51 ± 0.06	14.24***
WL	2.98 ± 0.04	2.85 ± 0.08	12.61***	2.30 ± 0.08	2.34 ± 0.08	1.74	2.66 ± 0.09	2.75 ± 0.09	14.25***

Variables	Colony 27			Colony 31			Colony 40		
	Queens n = 8	Workers n = 80	ANOVA (F)	Queens n = 11	Workers n = 52	ANOVA (F)	Queens n = 2	Workers n = 30	ANOVA (F)
HW	1.74 ± 0.03	1.74 ± 0.03	0.59	1.87 ± 0.04	1.88 ± 0.03	0.95	1.82 ± 0.01	1.85 ± 0.04	1.68
PML	0.62 ± 0.02	0.61 ± 0.02	0.12	0.68 ± 0.03	0.68 ± 0.02	0.24	0.66 ± 0.01	0.68 ± 0.02	1.98
MSW	1.10 ± 0.06	1.08 ± 0.04	0.76	1.14 ± 0.05	1.16 ± 0.09	0.34	1.08 ± 0.04	1.10 ± 0.04	1.14
T2BW	0.71 ± 0.03	0.70 ± 0.03	0.62	0.78 ± 0.04	0.79 ± 0.04	0.41	0.70 ± 0.04	0.74 ± 0.03	2.90
AL	2.07 ± 0.06	2.02 ± 0.22	0.19	2.26 ± 0.07	2.30 ± 0.06	2.24	2.15 ± 0.04	2.24 ± 0.07	3.62
T1BH	0.21 ± 0.02	0.20 ± 0.02	0.39	0.21 ± 0.02	0.21 ± 0.02	0.34	0.22 ± 0.03	0.22 ± 0.01	0.13
T1L	1.32 ± 0.05	1.34 ± 0.05	0.89	1.50 ± 0.05	1.52 ± 0.04	2.29	1.51 ± 0.03	1.50 ± 0.06	0.02
WL	2.37 ± 0.06	2.36 ± 0.05	0.37	2.71 ± 0.11	2.77 ± 0.07	2.71	2.70 ± 0.04	2.83 ± 0.09	3.42

\*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05

0.99 (in a scale that varies from 0 to 1 – where 0 indicates total discrimination, and 1 indicates no discrimination). The same result was confirmed after the classification test (Table 4) in which only colony 2 classified egglayers correctly in a 75% level.

**Color differences.** Size was not a good discriminator, but color seemed to give interesting clues for the social organization of *L. dorsata* (Fig 4). Workers and intermediates presented a pale color, but with several spots that varied from chestnut to dark brown. The color patterns in 52% of the egglayers were similar to those found in workers and intermediates. However, 42% of the egglayers were mostly yellow in their heads, thorax and abdomen (Fig 4). These differences were not related to age, because all yellow egglayers were old; but brown egglayers may be both young and old. Interestingly, yellow egglayers presented larger ovaries (pattern 4, see above) and darker egglayers smaller ovaries (pattern 3, see above).

## Discussion

In our study, *L. dorsata* only presented slightly morphologic differentiation between egglayers and workers.

Also, there is a physiological distinction found between egglayers and worker ovaries, except for the presence of a few females with ovarian development (but, see below), that may suggest strong control of reproduction. Group comparisons showed that egglayers are not a well-defined group based on size. In addition, Wilks' Lambda values were high demonstrating that in these colonies caste recognition is hardly done based on adult wasp morphology. In fact, castes morphological traits are always intersecting each other.

Even with slight morphologic differences, some egglayers of *L. dorsata* can be clearly separated by color as in *Protonectarina sylveirae* (Saussure) (Shima et al 1996) and other species of Epiponini (Richards 1978). Two patterns of color were found in *L. dorsata* egglayers, one that is yellow and bearing longer ovaries and other resembling workers in color and with smaller ovaries when compared to yellow egglayers. Because this color difference is so striking, we suggest that brown egglayers are, in fact, former workers that reached egglayer status (Mateus et al 2004).

As observed in *Synoeca* and *Metapolybia* (West-Eberhard 1978, 1981), young females, when orphaned by loss of the egglayers would lead the colony until new egglayers are produced or they may compete with those true egglayers during the early stages of the colony cycle. Our data support

Table 4 Classification matrix between observed and predicted classifications of groups after discriminant analysis in *Leipomeles dorsata*.

Colony	Observed classification	Predicted classification		Correctly classified females (%)
		Worker	Egglayer	
2	Worker	93	0	100
	Queen	1	3	75
25	Worker	1	62	98.4
	Queen	1	11	8.3
26	Worker	2	69	97.2
	Queen	5	10	33.3
27	Worker	80	0	100
	Queen	8	0	0
31	Worker	51	1	98.1
	Queen	10	1	9.1
40	Worker	30	0	100
	Queen	1	1	50

both hypotheses, because we found colonies with both kinds of egg-layers. In only one colony, however, yellow egg-layers were absent and only two brown egg-layers were found. Because these females always bear small ovaries, it is possible that they may function as replacements, leading the colony to a new sexual production. Another interesting fact is that in the presence of a very low number of yellow egg-layers, there are more brown egg-layers and some intermediates (that might simply be brown, but uniseminated egg-layers). On the other hand, in the presence of more yellow egg-layers there are a few brown egg-layers and no intermediates.

Low morphological differentiation accompanied by high physiological distinction was found in *Asteloeca* (Raw), *Synoecca* and *Metapolybia* (Baio *et al* 2003, Noll *et al* 2004). During cyclical oligogyny, a low number of egg-layers would signal new females to become new egg-layers (West-Eberhard 1978, Strassmann *et al* 1991). After egg-layers re-population, orphanage behaviors cease and workers use aggressive behaviors to inhibit other new females from becoming egg-layers (Baio *et al* 2003). In fact, we found only worker-like egg-layers in colony 40, reinforcing the assumption that new reproductives are produced only during discrete windows of time; however, young workers would be some kind of “backup” egg-layers. Even though this may sound odd, a similar strategy is found in the Neotropical



Fig 4 Color discrimination among egg-layers (a,b,c), intermediate (d,e,f) and workers (g,h,i) of *Leipomeles dorsata* in the analyzed colonies.

bumble bee *Bombus atratus* (Silva-Matos & Garófalo 1996), in which queens may be killed by workers and some inseminated workers assume reproduction in the colony until the production of new reproductives.

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### References

- Alexander R D (1974) The evolution of social behavior. *Annu Rev Ecol Syst* 5: 325-383.
- Anderson M (1984) The evolution of eusociality. *Annu Rev Ecol Syst* 15: 165-189.
- Baio M V, Noll F B, Zucchi R (2003) Shape differences rather than size differences between castes in the Neotropical swarm founding wasp *Metapolybia docilis* (Hymenoptera: Vespidae, Epiponini). *BMC Evol Biol* 3: 10.
- Bourke A F G (1988) Worker reproduction in the higher eusocial Hymenoptera. *Q Rev Biol* 63: 291-311.
- Bourke A F G (1998) Sex ratios in bumblebees. *Philos Trans R Soc Lond B Biol Sci* 352: 1921-1933.
- Brockmann H J (1984) The evolution of social behaviour in Insects, p.340-361. In Krebs J R, Davies N B (eds) *Behavioural ecology: an evolutionary approach*. 2nd ed. Blackwell, Oxford, 484p.
- Carpenter J M (2004) Synonymy of the genus *Marimbonda* Richards, 1978, with *Leipomeles* Möbius, 1856 (Hymenoptera: Vespidae; Polistinae) and a new key to the genera of paper wasps of the New World. *Am Mus Novit* 3465: 16p.
- Hamilton W D (1964a) The genetical evolution of social behaviour. *J Theor Biol* 7: 1-16.
- Hamilton W D (1964b) The genetical evolution of social behaviour. *J Theor Biol* 7: 17-52.
- Jeanne R L (1991) The swarm-founding Polistinae, p.191-231. In Ross K G, Matthews R W (eds) *The social biology of wasps*, Ithaca, New York, Cornell Univ Press, 678p.
- Mateus S, Noll F B, Zucchi R (2004) Caste flexibility and variation according to the colony cycle in the swarm-founding wasp, *Parachartergus fraternus* (Hymenoptera: Vespidae: Epiponini). *J Kansas Entomol Soc* 77: 281-294.
- Noll F B, Wenzel J W, Zucchi R (2004) Evolution of caste in neotropical swarm-founding wasps (Hymenoptera: Vespidae: Epiponini). *Am Mus Novit* 3467: 24.
- Noll F B, Wenzel J W (2008) Caste in the swarming wasps: 'queenless' societies in highly social insects. *Biol J Linn Soc* 93: 509-522.
- O'Donnell S (1998) Reproductive caste determination in eusocial wasps (Hymenoptera: Vespidae). *Annu Rev Entomol* 43: 323-46.
- Richards O W (1971) The biology of the social wasps (Hymenoptera: Vespidae). *Biol Rev* 46: 483-528.
- Richards O W (1978) The social wasps of the Americas excluding the Vespinae. *British Museum (Natural History)*, London, 580p.
- Shima S N, Yamane S, Zucchi R (1996) Morphological caste differences in some Neotropical swarm-founding polistine wasps III. *Protonectarina sylveirae* (Hymenoptera, Vespidae). *Bull Fac Ed Ibaraki Uni* 45: 57-67.
- Silva-Matos E V, Garófalo C A (1996) Observations on the development of queenless colonies of *Bombus atratus* (Hymenoptera, Apidae). *J Apic Res* 34: 177-185.
- Strassmann J E, Queller D C, Solis C R, Hughes C R (1991) Relatedness and queen number in the Neotropical wasp, *Parachartergus colobopterus*. *Anim Behav* 42: 461-470.
- West-Eberhard M J (1973) Monogyny in polygynous social wasps. In *Proceedings of the VII Congress of I.U.S.S.I.* London, p.396-403.
- West-Eberhard M J (1975) The evolution of social behavior by kin selection. *Q Rev Biol* 50: 1-33.
- West-Eberhard M J (1978) Temporary queens in *Metapolybia* wasps; non-reproductive helpers without altruism? *Science* 200: 441-443.
- West-Eberhard M J (1981) Intragroup selection and the evolution of insect societies, p.3-17. In Alexander R D, Tinkle D W (eds) *Natural selection and social behaviour*, New York, Chiron Press, 532p.
- Zucchi R (1993) Ritualized dominance, evolution of queenworker interactions and related aspects in stingless bees (Hymenoptera: Apidae), p.207-249. In Inoue T, Yamane S (eds) *Evolution of insect societies*, Tokyo, Hakuhinsha Press, 300p.

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